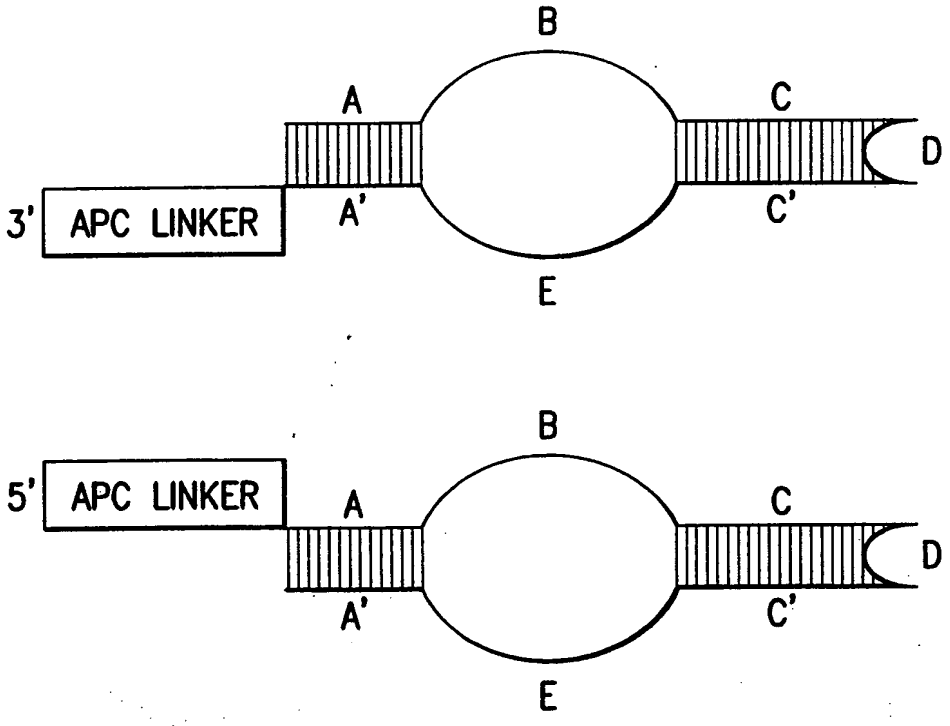


FIG.1

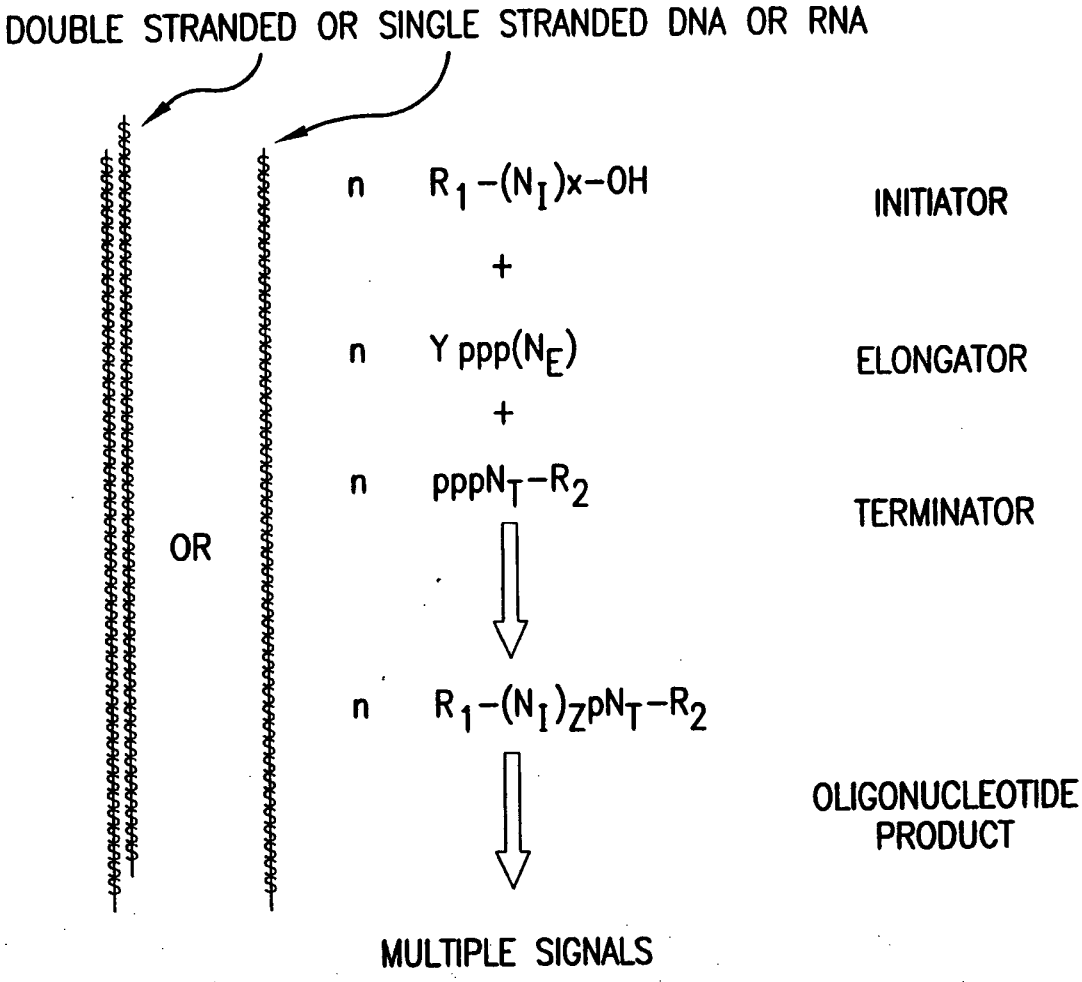
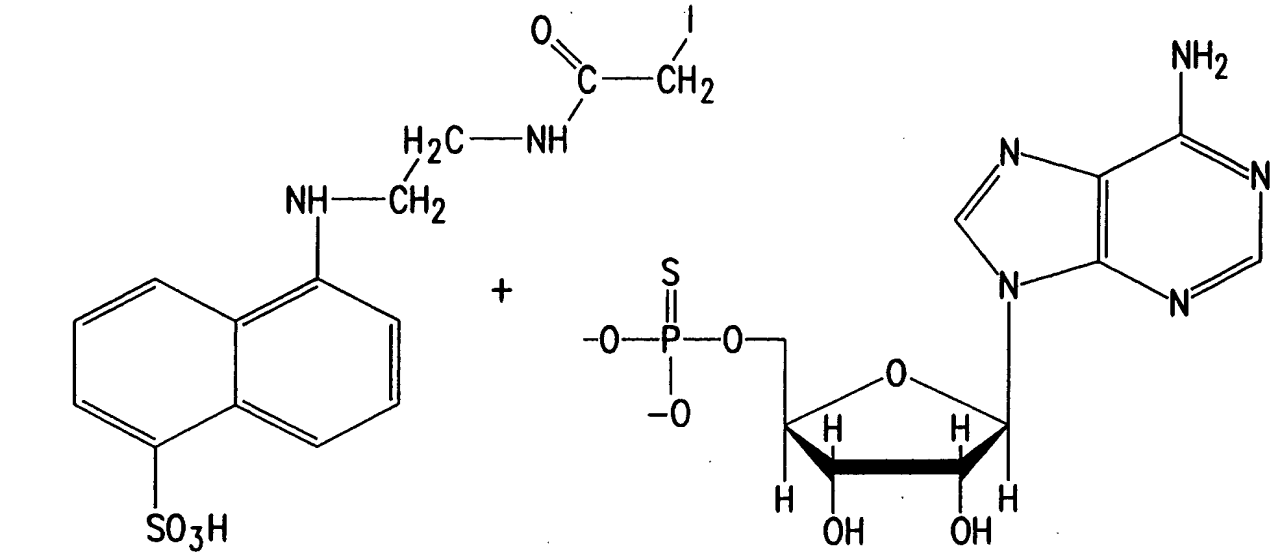
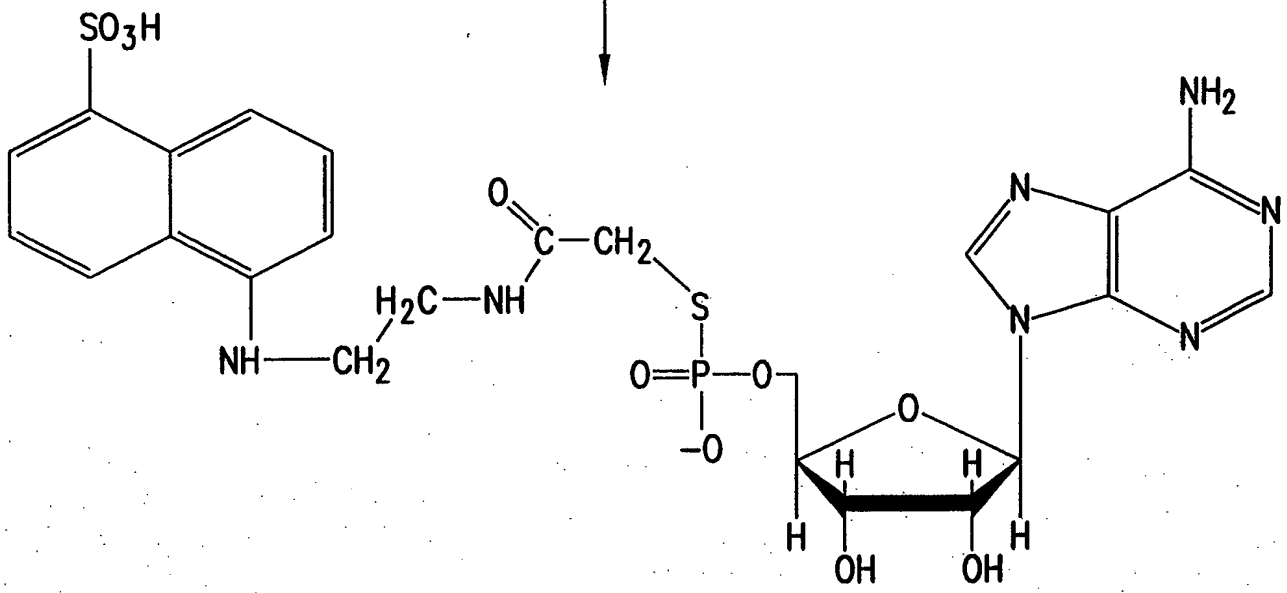

DOUBLE STRANDED OR SINGLE STRANDED DNA OR RNA
OR
n $R_1-(N_I)x-OH$
INITIATOR
+
n $Y\,ppp(N_E)$
ELONGATOR
+
n $pppN_T-R_2$
TERMINATOR
n $R_1-(N_I)_Z pN_T-R_2$
OLIGONUCLEOTIDE PRODUCT
MULTIPLE SIGNALS

FIG.2



IAEDANS AMPS



5' AEDANS-S-AMP

FIG.3

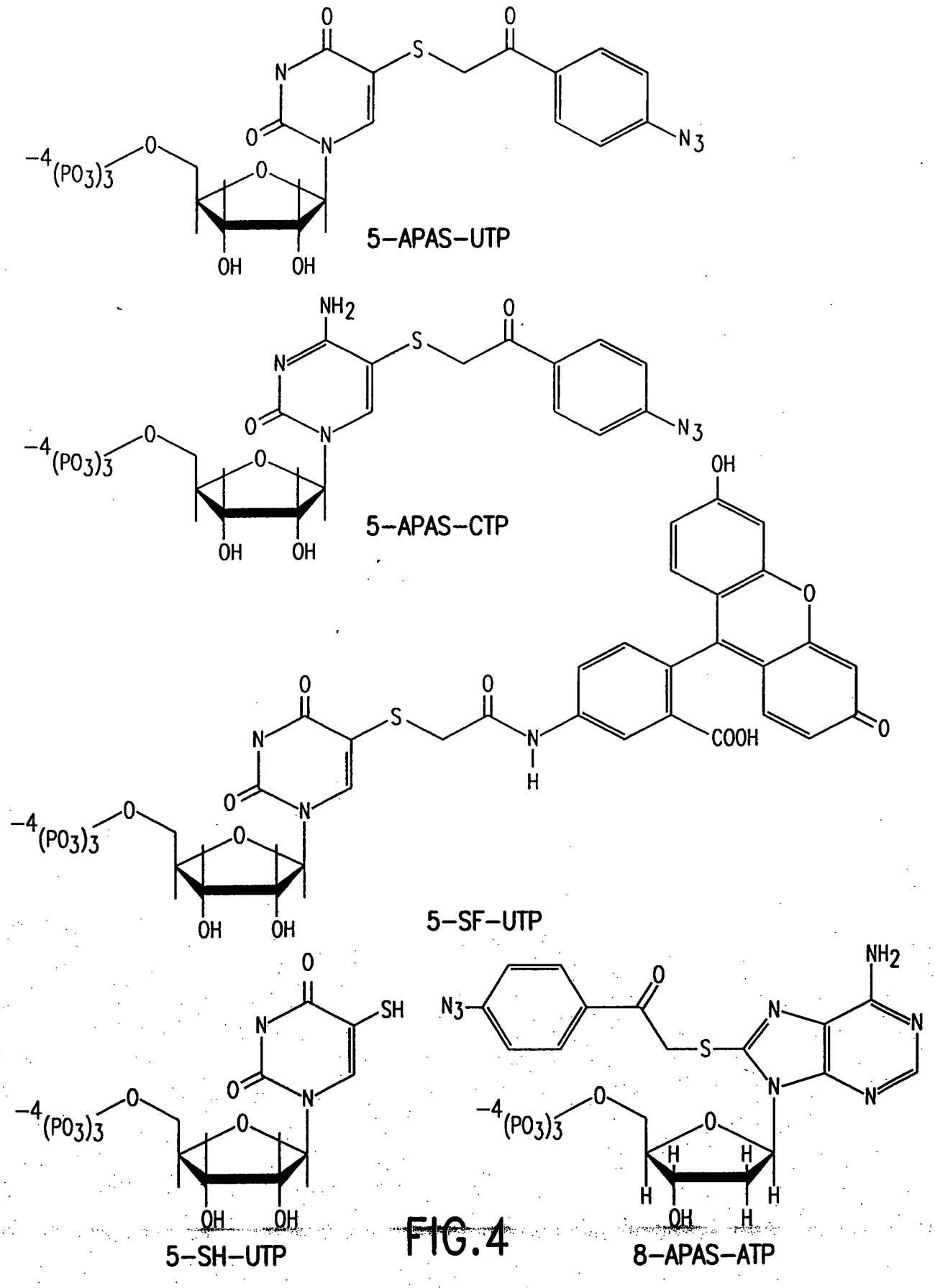
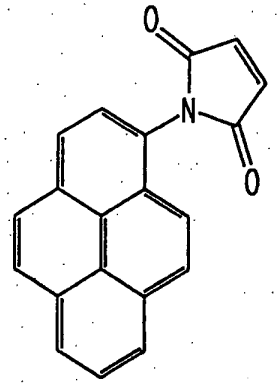
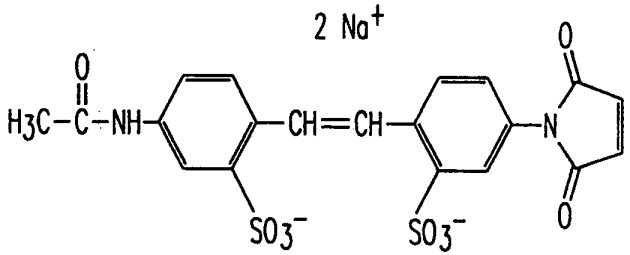


FIG.4

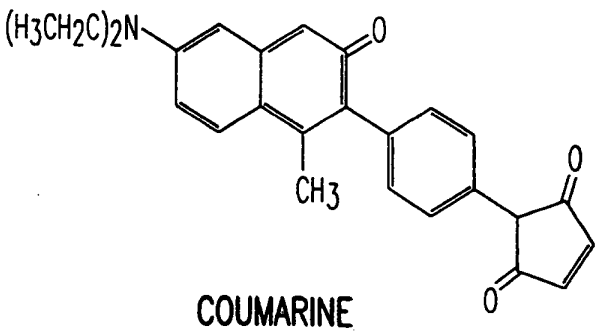
Appl. No. *To Be Assigned*; Group Art Unit: *To Be Assigned*; Inventors: Michelle M. Hanna.; Tel: 202.371-2600
Title: Molecular Detection Systems Utilizing Reiterative Oligonucleotide Synthesis



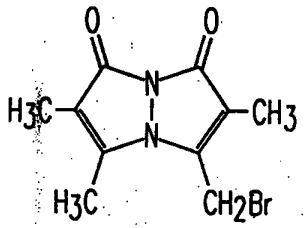
PYRENE



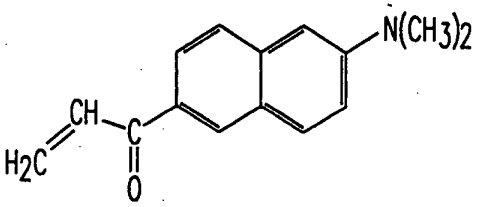
STILBENE



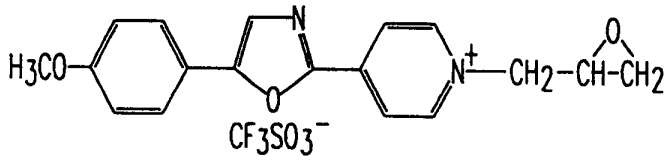
COUMARINE



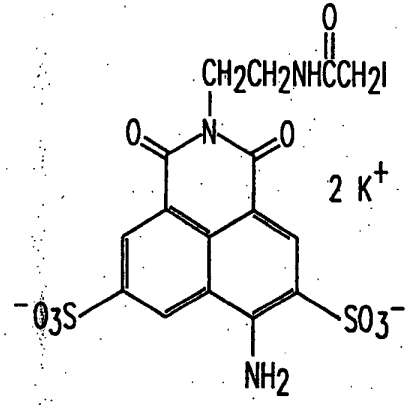
BIMANE



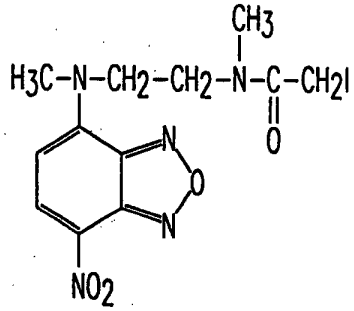
NAPHTHALENE



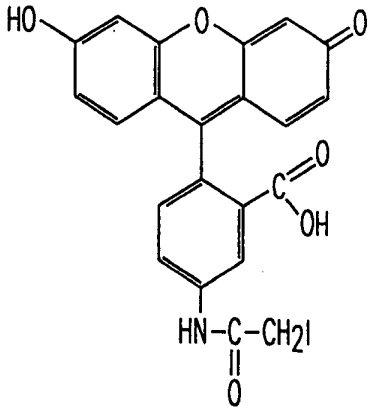
PYRIDYLOXAZOLE



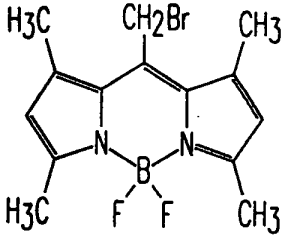
NAPHTHALIMIDE



NBD



FLUORESCEIN



BODIPY™

FIG.5

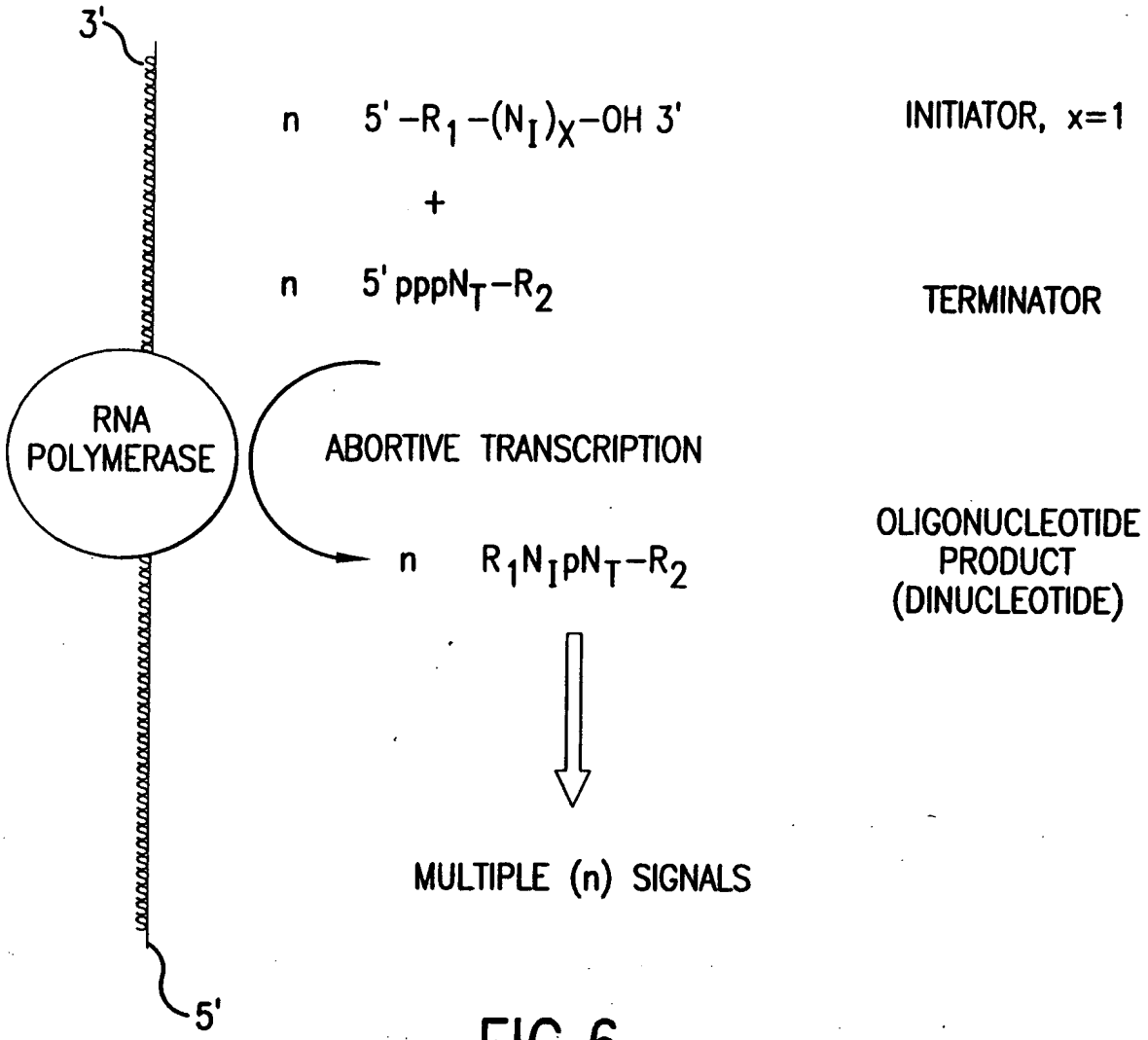

3'
n 5'-R1-(NI)X-OH 3'
INITIATOR, x=1
+
n 5' pppNT-R2
TERMINATOR
RNA POLYMERASE
ABORTIVE TRANSCRIPTION
n R1NIpNT-R2
OLIGONUCLEOTIDE PRODUCT (DINUCLEOTIDE)
MULTIPLE (n) SIGNALS
5'

FIG.6

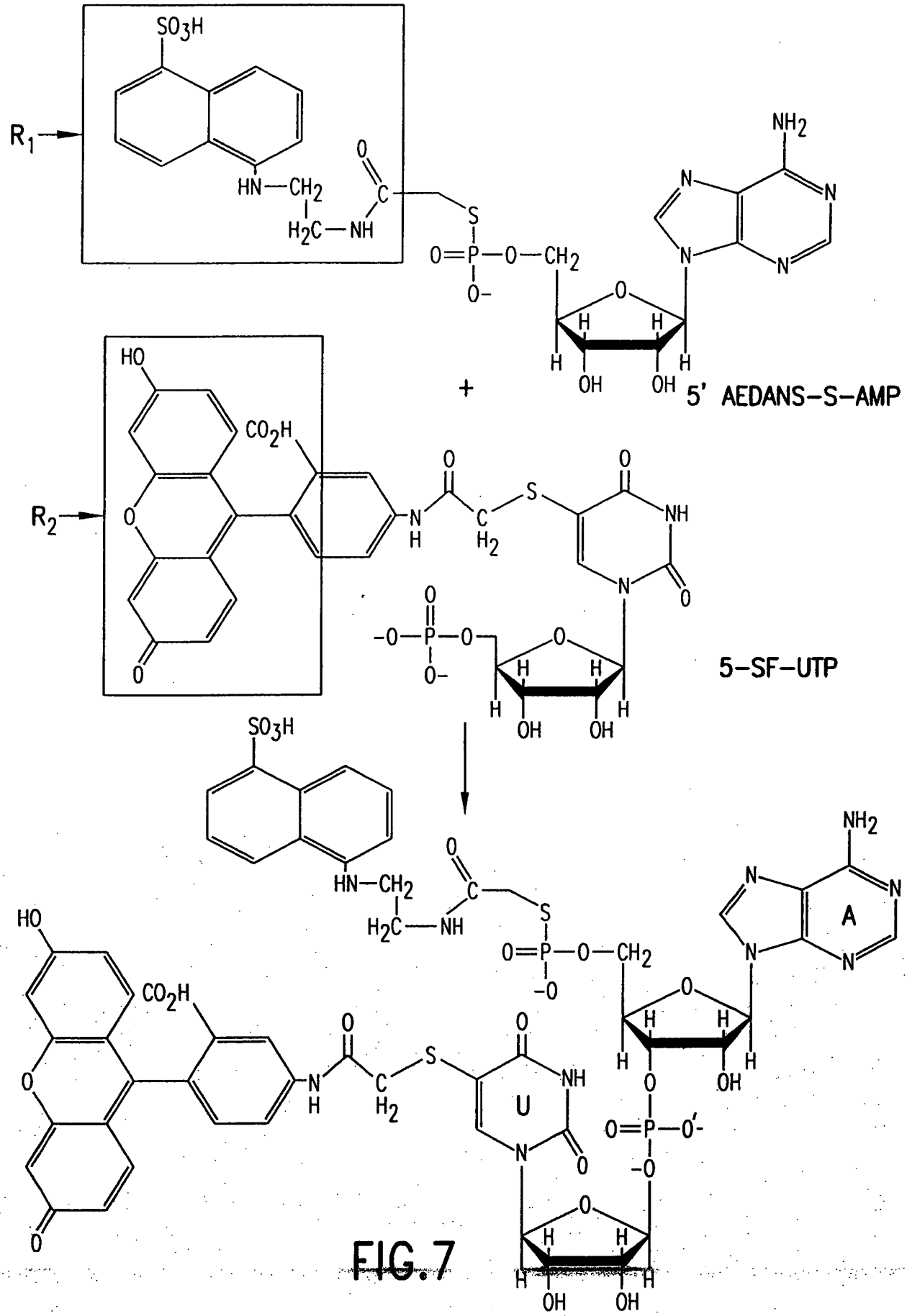


FIG. 7

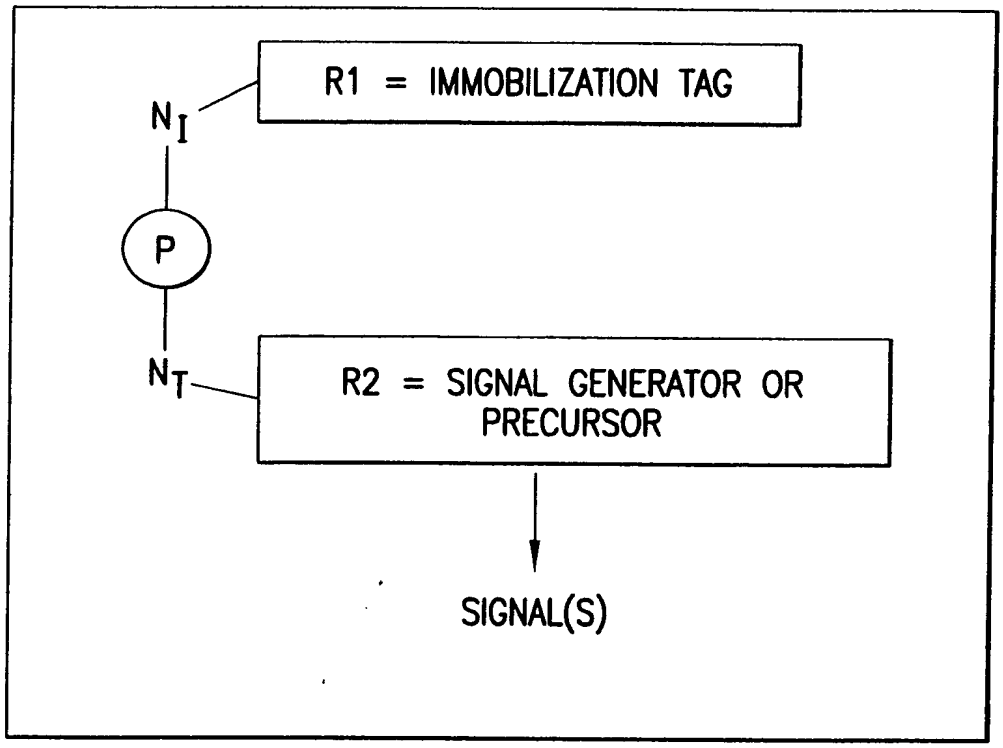

R1 = IMMOBILIZATION TAG
N_I
P
N_T
R2 = SIGNAL GENERATOR OR PRECURSOR
SIGNAL(S)

FIG.8

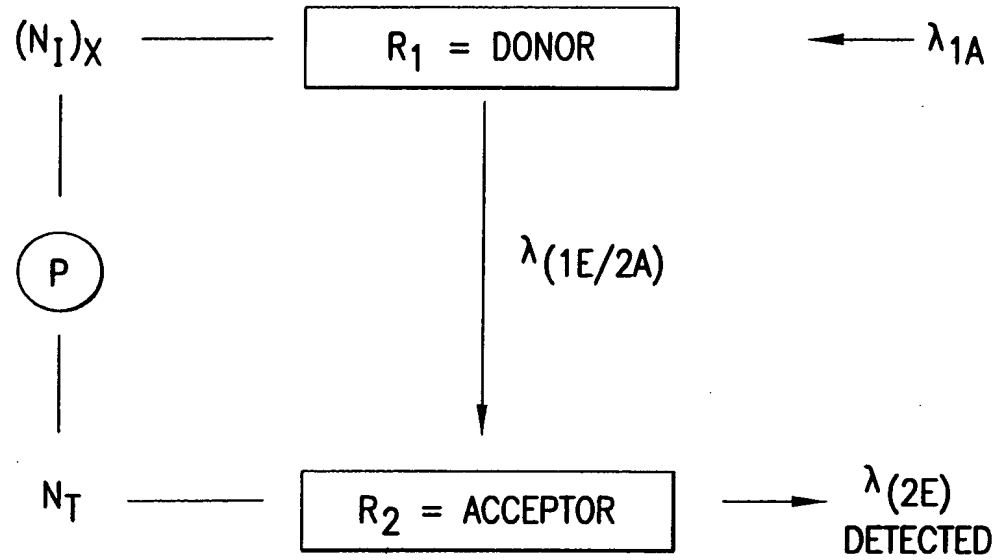

$(N_I)_X$
R_1 = DONOR
λ_{1A}
P
λ(1E/2A)
N_T
R_2 = ACCEPTOR
λ(2E)
DETECTED

FIG.9

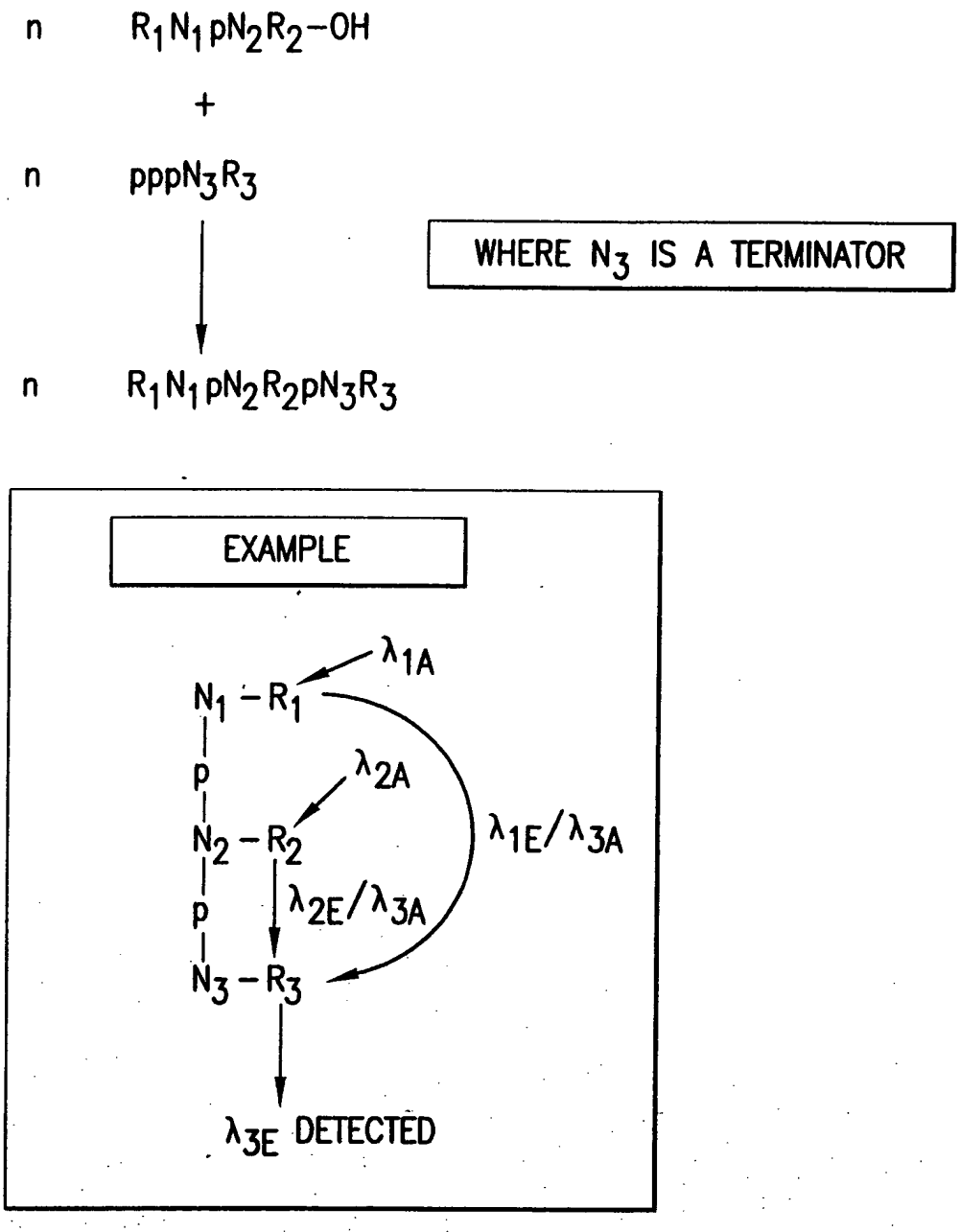

n R1N1pN2R2–OH
+
n pppN3R3
n R1N1pN2R2pN3R3
WHERE N3 IS A TERMINATOR
EXAMPLE
λ1A
N1 – R1
p
λ2A
N2 – R2
λ1E/λ3A
p
λ2E/λ3A
N3 – R3
λ3E DETECTED

FIG.10

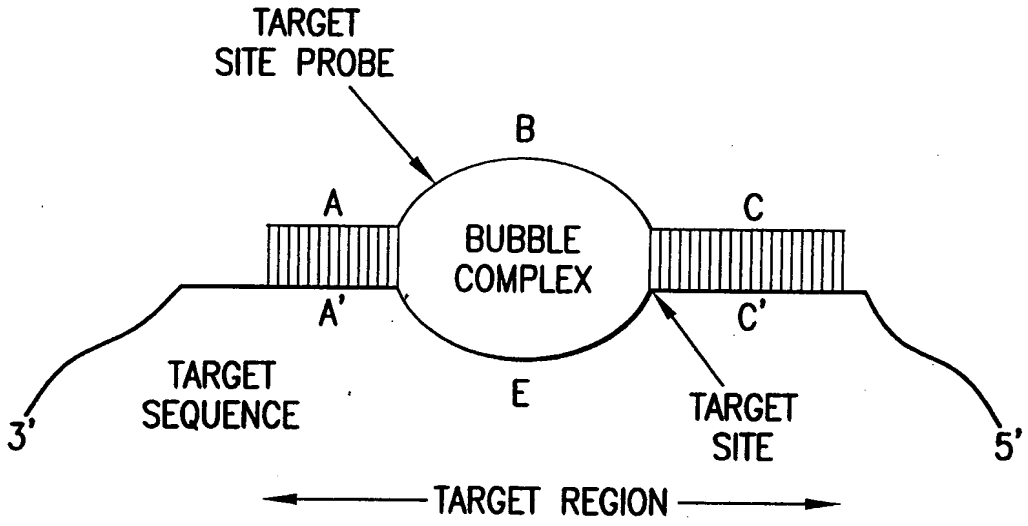

TARGET
SITE PROBE
B
A
C
BUBBLE
COMPLEX
A'
C'
TARGET
SEQUENCE
E
TARGET
SITE
3'
5'
TARGET REGION

FIG.11

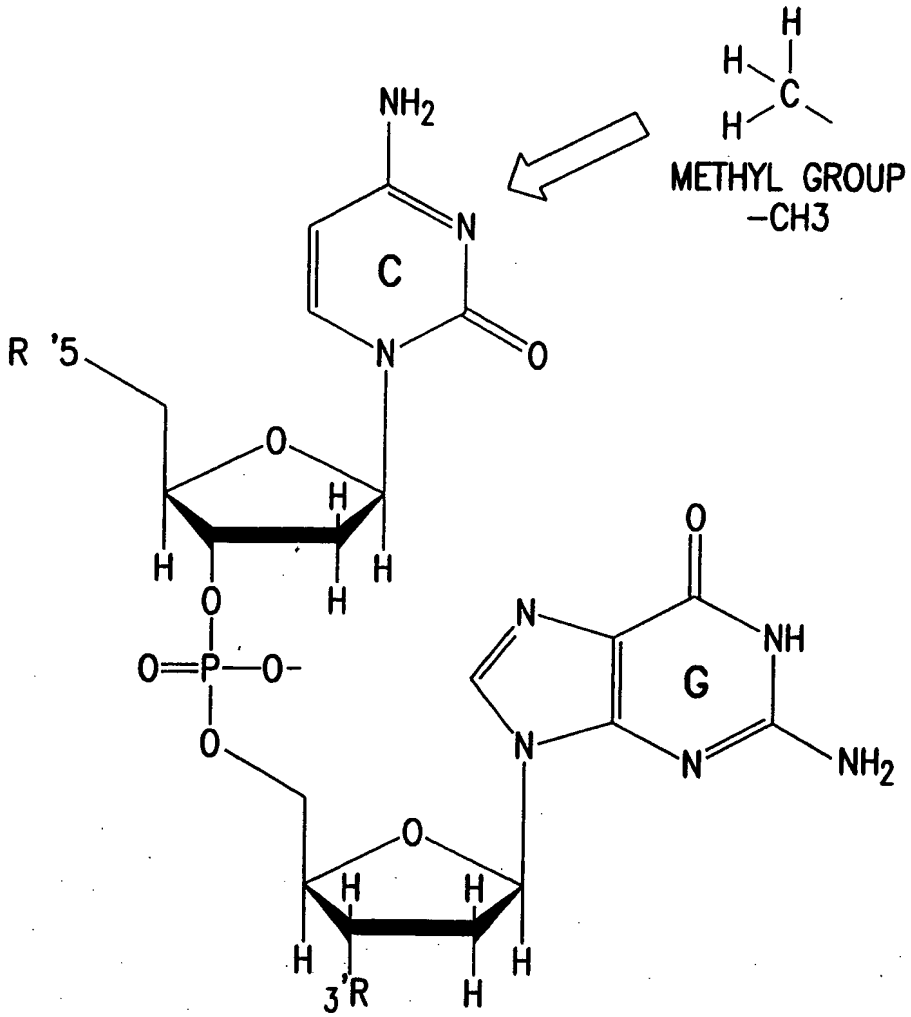


FIG.12

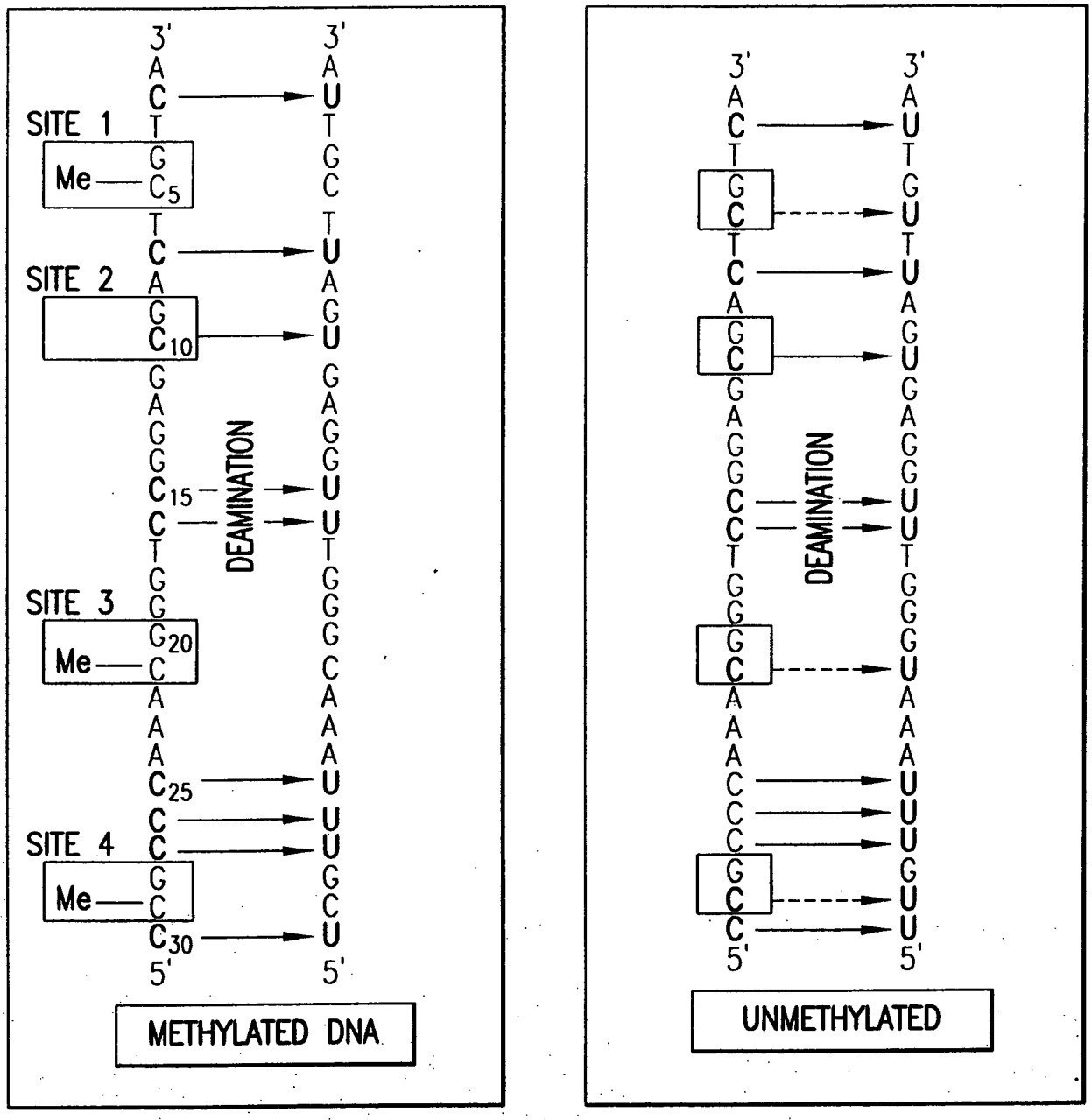

SITE 1
Me
SITE 2
SITE 3
Me
SITE 4
Me
DEAMINATION
METHYLATED DNA
DEAMINATION
UNMETHYLATED

FIG.13

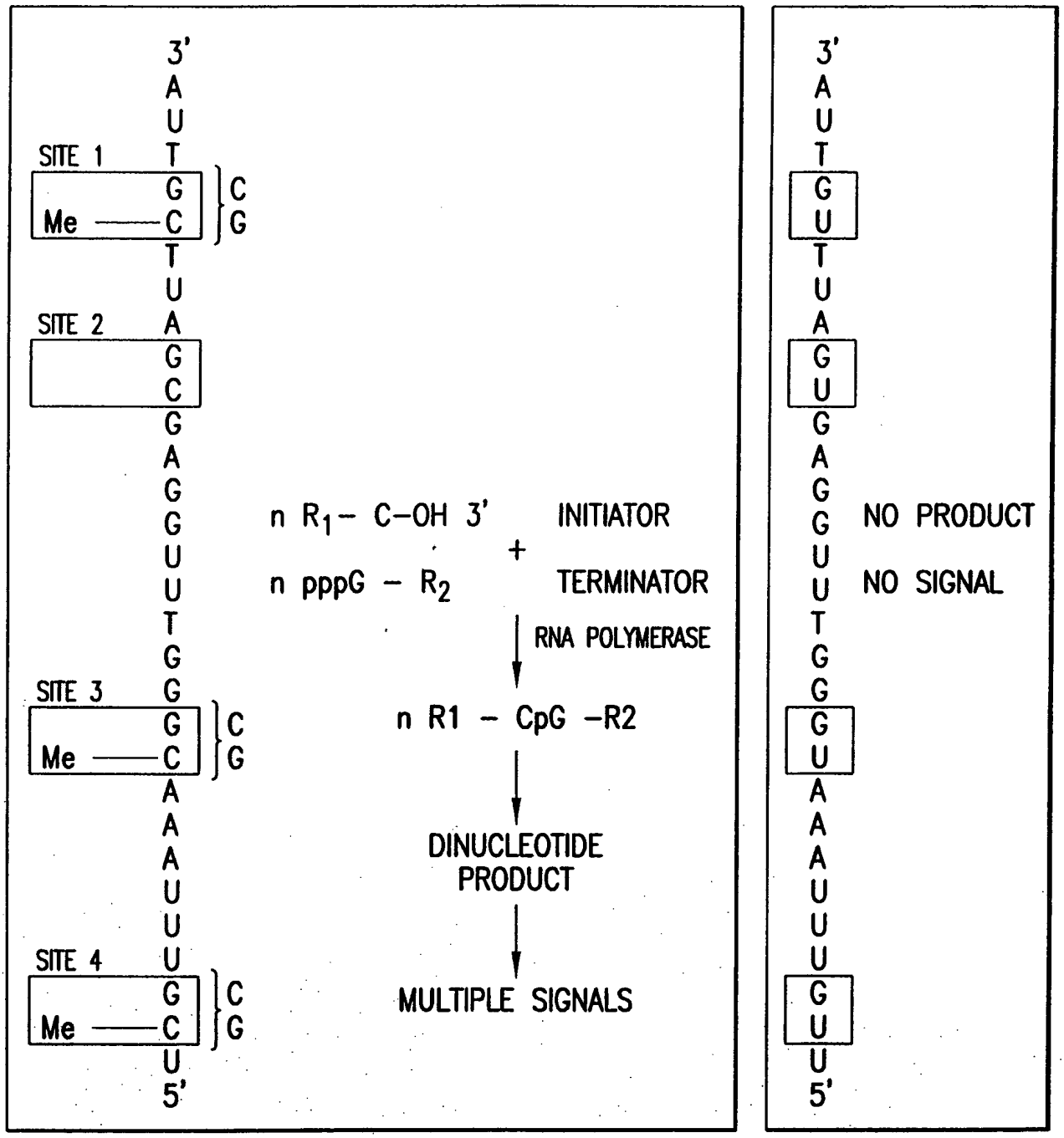

3'
A
U
T
SITE 1
G
Me
C
C
G
T
U
A
SITE 2
G
C
G
A
G
G
U
U
T
G
G
SITE 3
G
Me
C
C
G
A
A
A
U
U
U
SITE 4
G
Me
C
C
G
U
5'
n R1 – C–OH 3'
INITIATOR
+
n pppG – R2
TERMINATOR
RNA POLYMERASE
n R1 – CpG –R2
DINUCLEOTIDE PRODUCT
MULTIPLE SIGNALS
NO PRODUCT
NO SIGNAL
DEAMINATED METHYLATED DNA
DEAMINATED UNMETHYLATED DNA

FIG.14

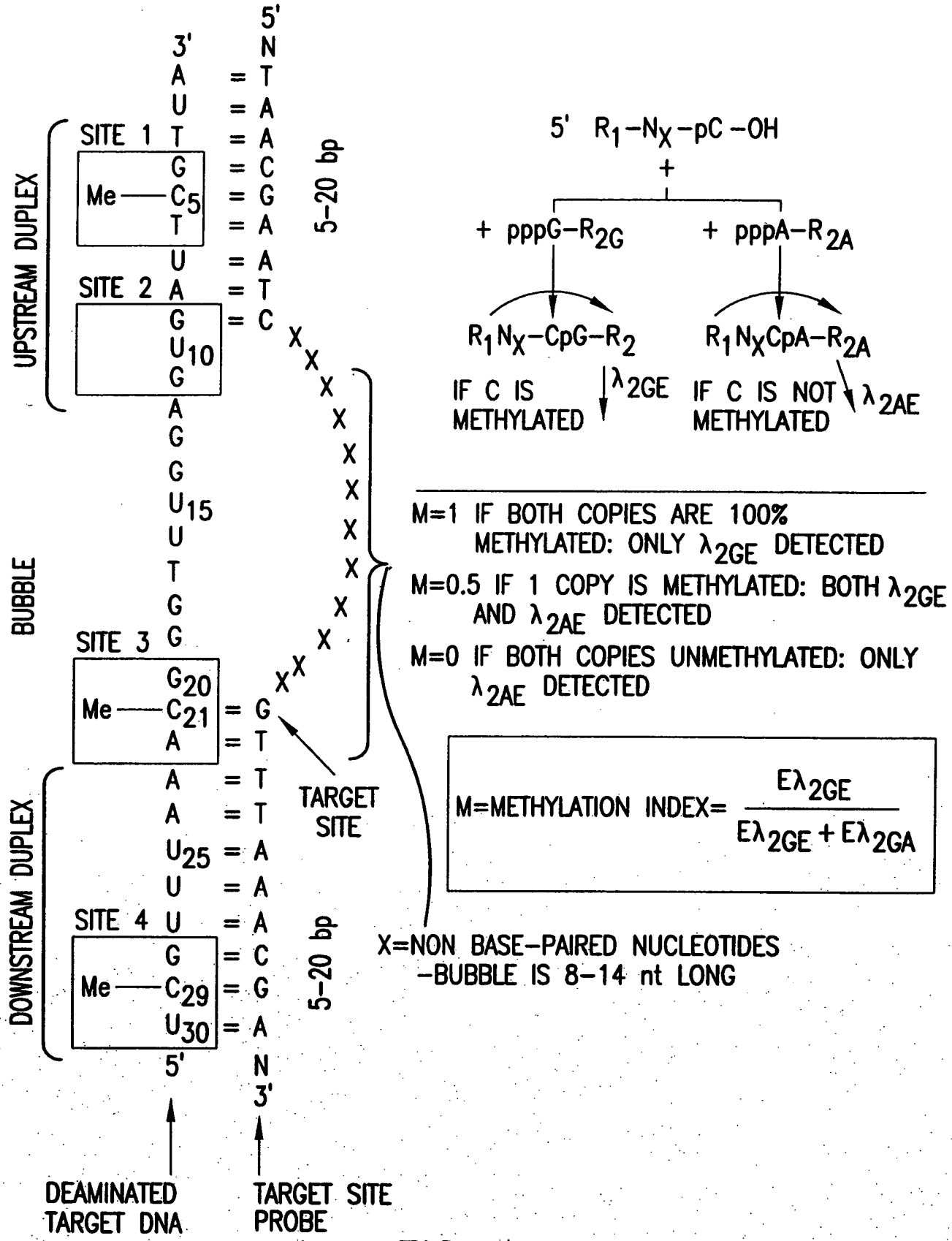

5'
3'
N
A = T
U = A
T = A
G = C
Me — C5 = G
T = A
U = A
SITE 1
SITE 2
A = T
G = C
U10
G
A
G
G
U15
U
T
G
G
SITE 3
G20
Me — C21 = G
A = T
A = T
A = T
U25 = A
U = A
SITE 4
U = A
G = C
Me — C29 = G
U30 = A
5'
N
3'
UPSTREAM DUPLEX
BUBBLE
DOWNSTREAM DUPLEX
5–20 bp
5–20 bp
X X X X X X X X X X X X X X
TARGET SITE
DEAMINATED TARGET DNA
TARGET SITE PROBE
5' R1–NX–pC–OH
+
+ pppG–R2G
+ pppA–R2A
R1NX–CpG–R2
R1NXCpA–R2A
IF C IS METHYLATED
λ2GE
IF C IS NOT METHYLATED
λ2AE
M=1 IF BOTH COPIES ARE 100% METHYLATED: ONLY λ2GE DETECTED
M=0.5 IF 1 COPY IS METHYLATED: BOTH λ2GE AND λ2AE DETECTED
M=0 IF BOTH COPIES UNMETHYLATED: ONLY λ2AE DETECTED
M=METHYLATION INDEX= Eλ2GE / Eλ2GE + Eλ2GA
X=NON BASE-PAIRED NUCLEOTIDES
-BUBBLE IS 8–14 nt LONG

FIG.15

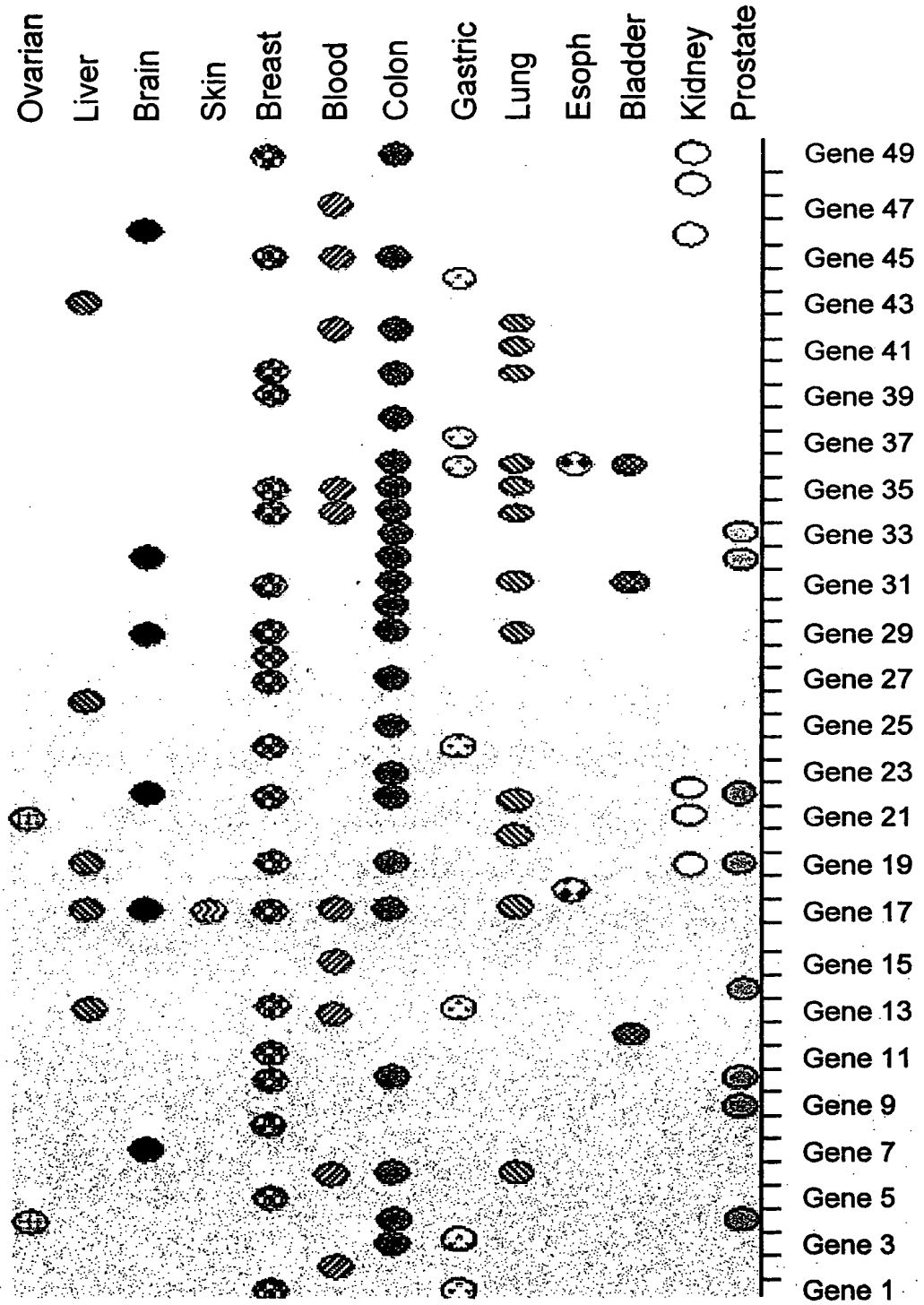

Ovarian
Liver
Brain
Skin
Breast
Blood
Colon
Gastric
Lung
Esoph
Bladder
Kidney
Prostate
Gene 49
Gene 47
Gene 45
Gene 43
Gene 41
Gene 39
Gene 37
Gene 35
Gene 33
Gene 31
Gene 29
Gene 27
Gene 25
Gene 23
Gene 21
Gene 19
Gene 17
Gene 15
Gene 13
Gene 11
Gene 9
Gene 7
Gene 5
Gene 3
Gene 1

FIG. 16

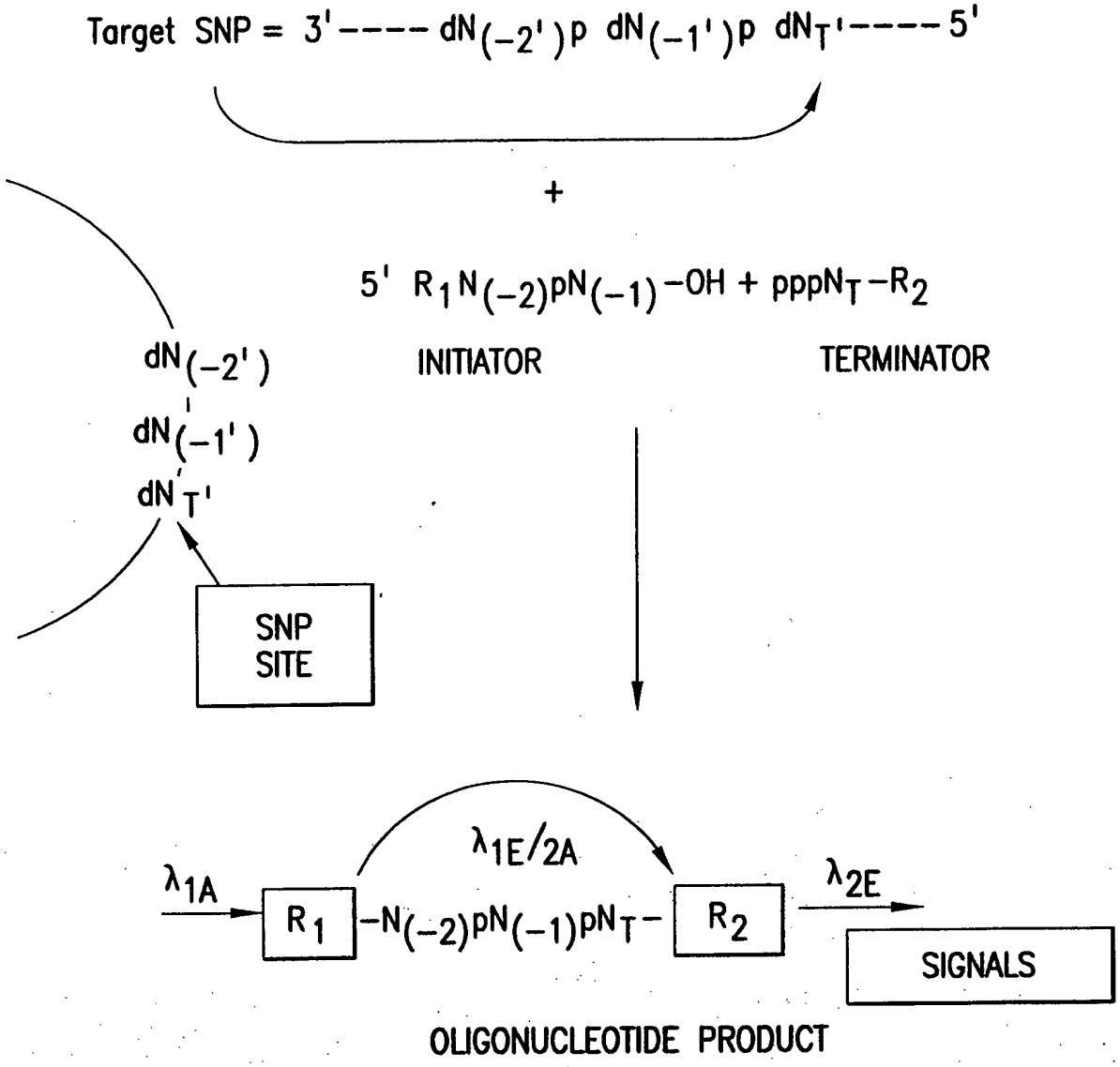

Target SNP = 3'---- dN(-2')p dN(-1')p dNT'---- 5'
+
5' R1N(-2)pN(-1)-OH + pppNT-R2
INITIATOR
TERMINATOR
dN(-2')
dN(-1')
dNT'
SNP SITE
λ1E/2A
λ1A
R1
-N(-2)pN(-1)pNT-
R2
λ2E
SIGNALS
OLIGONUCLEOTIDE PRODUCT

FIG.17

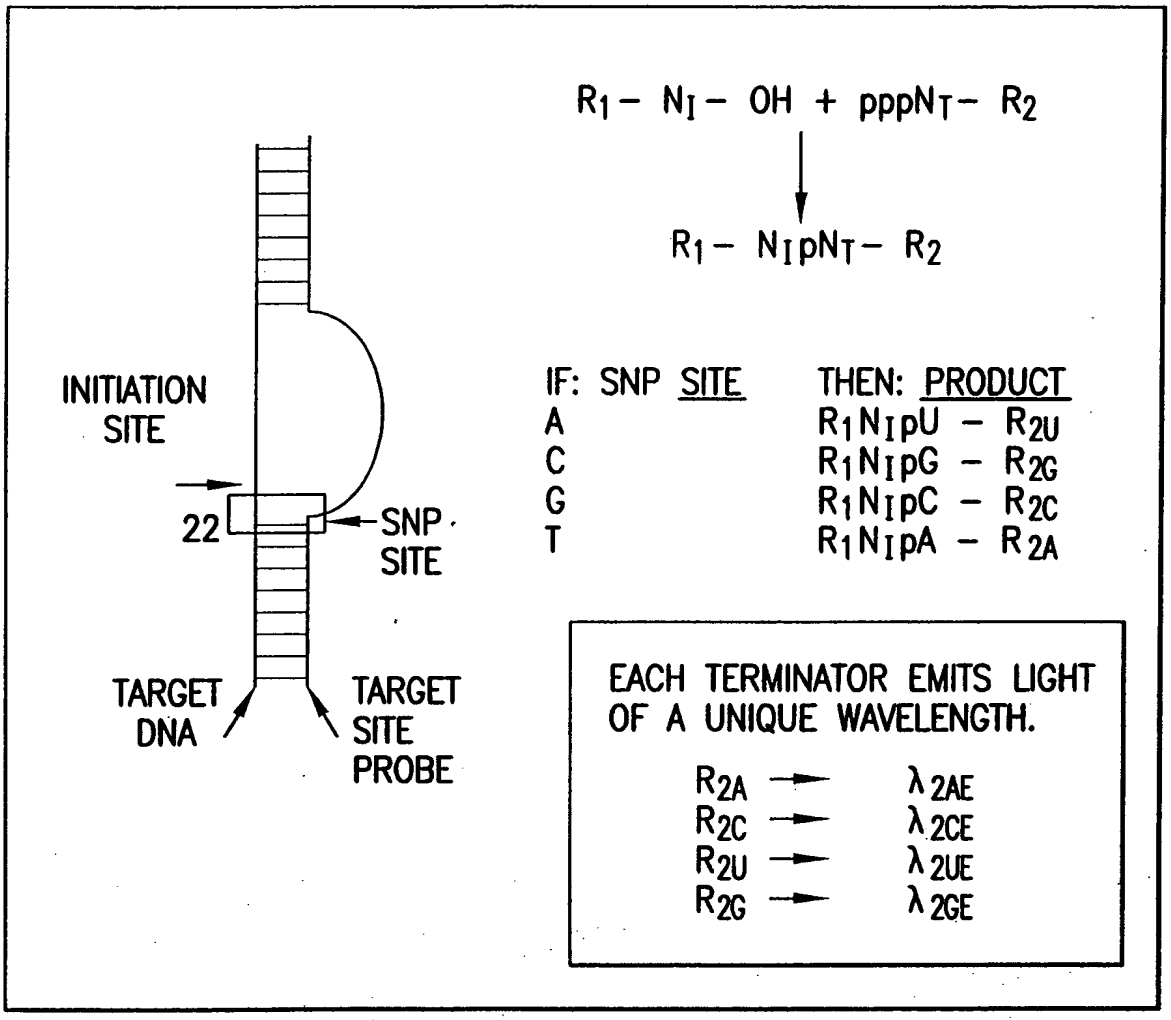
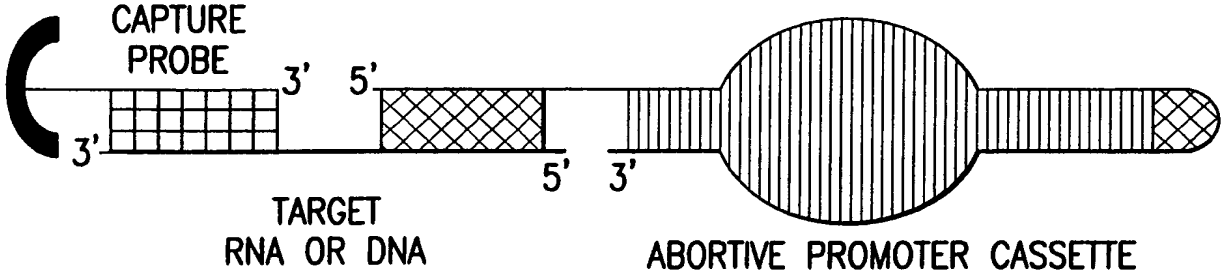

$R_1 - N_I - OH + pppN_T - R_2$
$R_1 - N_I pN_T - R_2$
INITIATION SITE
22
SNP SITE
TARGET DNA
TARGET SITE PROBE
IF: SNP SITE
A
C
G
T
THEN: PRODUCT
$R_1N_IpU - R_{2U}$
$R_1N_IpG - R_{2G}$
$R_1N_IpC - R_{2C}$
$R_1N_IpA - R_{2A}$
EACH TERMINATOR EMITS LIGHT OF A UNIQUE WAVELENGTH.
$R_{2A} \rightarrow \lambda_{2AE}$
$R_{2C} \rightarrow \lambda_{2CE}$
$R_{2U} \rightarrow \lambda_{2UE}$
$R_{2G} \rightarrow \lambda_{2GE}$

FIG.18


CAPTURE PROBE
3'
5'
3'
5'
3'
TARGET RNA OR DNA
ABORTIVE PROMOTER CASSETTE

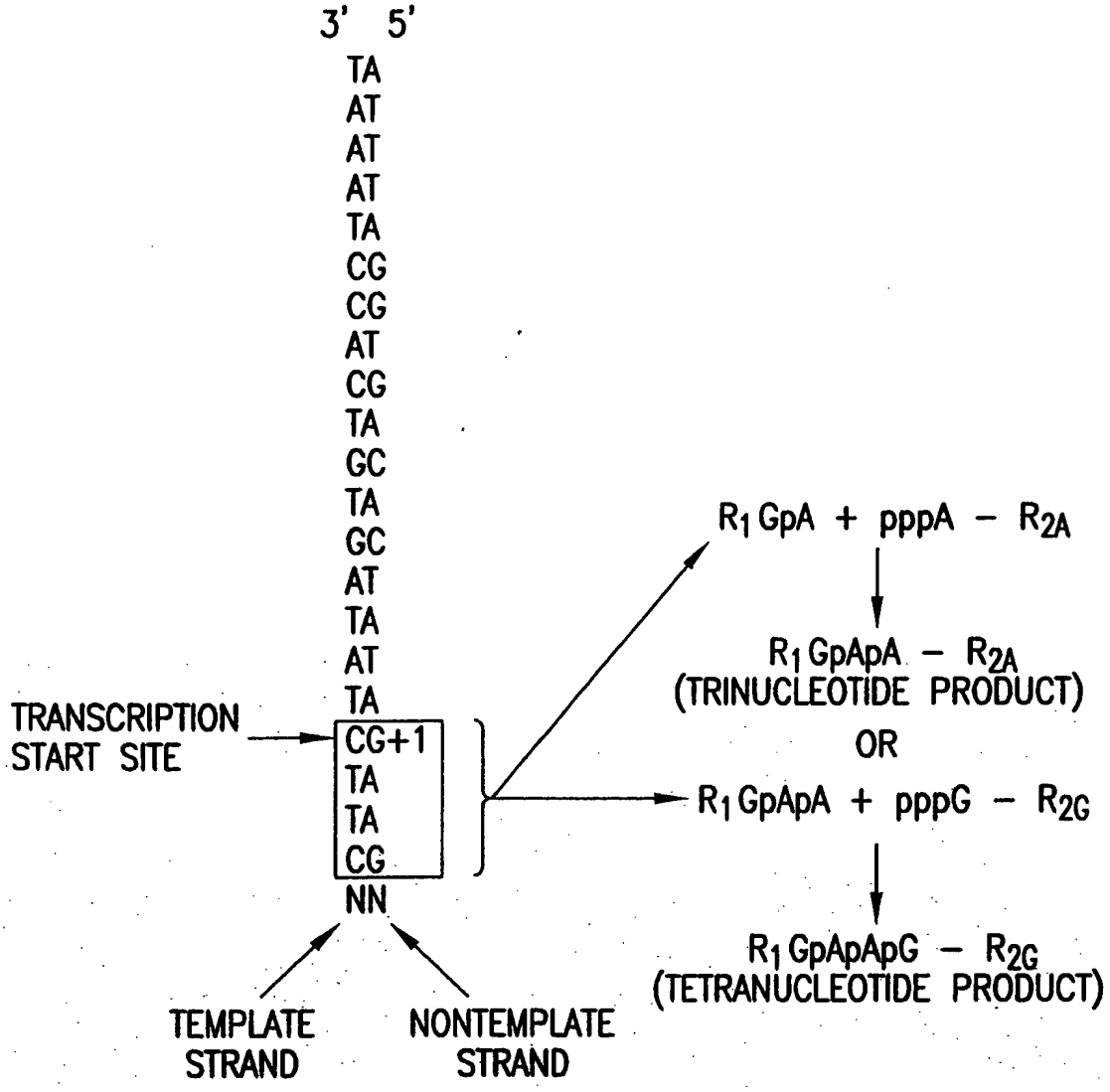

3' 5'
TA
AT
AT
AT
TA
CG
CG
AT
CG
TA
GC
TA
GC
AT
TA
AT
TA
TRANSCRIPTION START SITE
CG+1
TA
TA
CG
NN
TEMPLATE STRAND
NONTEMPLATE STRAND
R1 GpA + pppA – R2A
R1 GpApA – R2A
(TRINUCLEOTIDE PRODUCT)
OR
R1 GpApA + pppG – R2G
R1 GpApApG – R2G
(TETRANUCLEOTIDE PRODUCT)

FIG.19

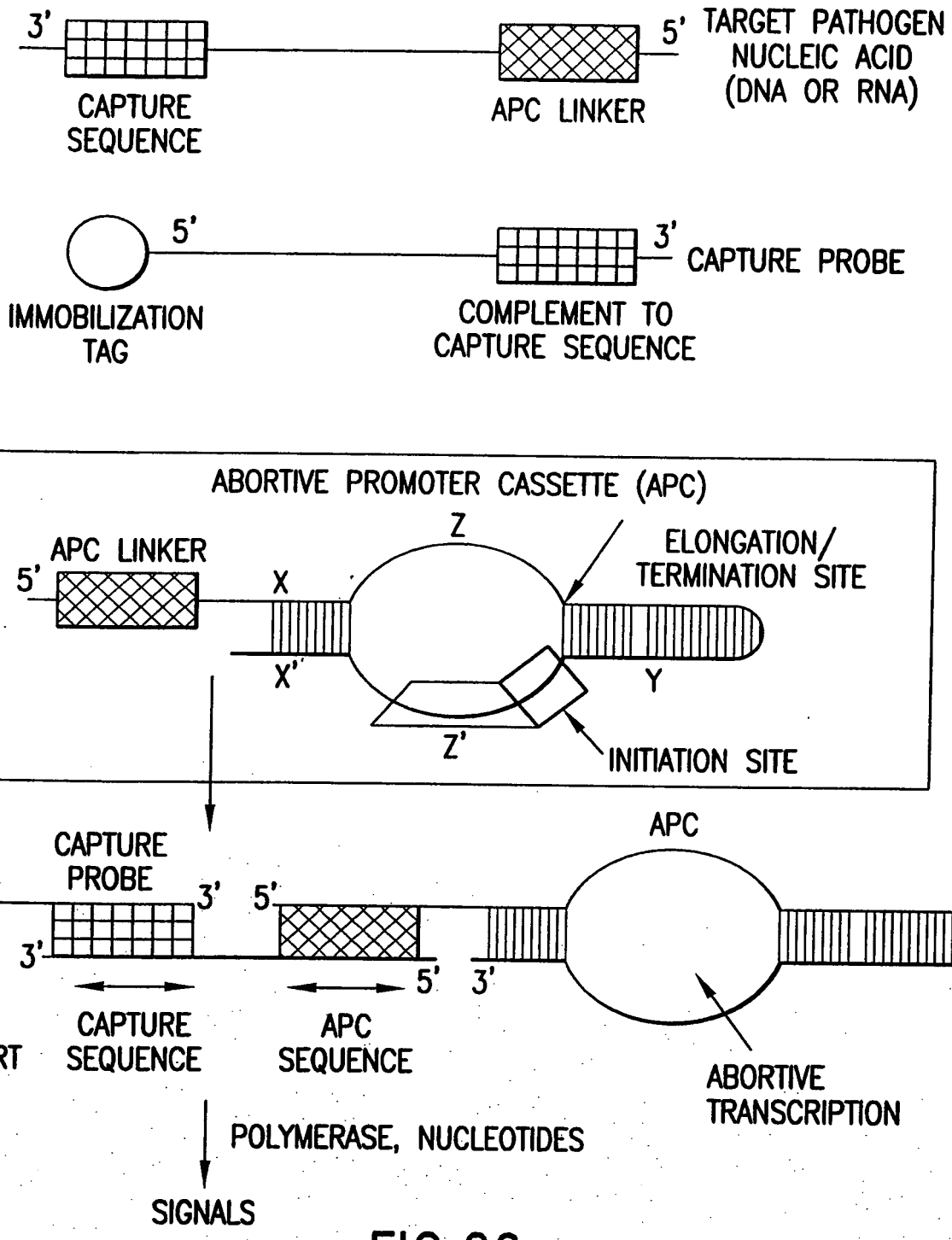

3'
CAPTURE SEQUENCE
APC LINKER
5'
TARGET PATHOGEN NUCLEIC ACID (DNA OR RNA)
5'
IMMOBILIZATION TAG
3'
CAPTURE PROBE
COMPLEMENT TO CAPTURE SEQUENCE
ABORTIVE PROMOTER CASSETTE (APC)
APC LINKER
5'
X
X'
Z
Z'
ELONGATION/ TERMINATION SITE
Y
INITIATION SITE
CAPTURE PROBE
3'
5'
3'
5'
3'
APC
SOLID SUPPORT
CAPTURE SEQUENCE
APC SEQUENCE
ABORTIVE TRANSCRIPTION
POLYMERASE, NUCLEOTIDES
SIGNALS

FIG.20

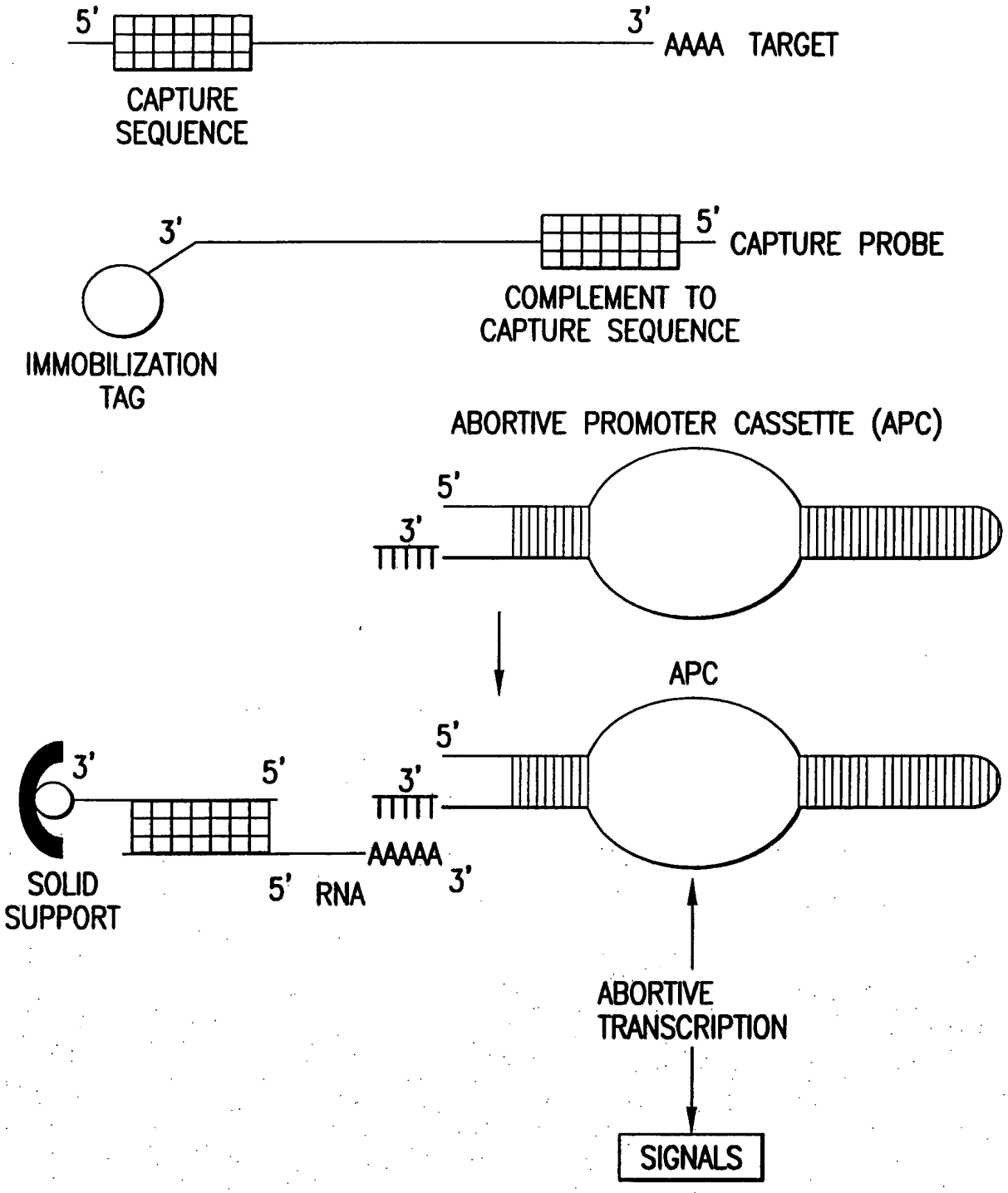

5'
3'
AAAA TARGET
CAPTURE SEQUENCE
3'
5'
CAPTURE PROBE
COMPLEMENT TO CAPTURE SEQUENCE
IMMOBILIZATION TAG
ABORTIVE PROMOTER CASSETTE (APC)
5'
3'
TTTTT
APC
5'
3'
TTTTT
3'
5'
AAAAA
SOLID SUPPORT
5' RNA
3'
ABORTIVE TRANSCRIPTION
SIGNALS

FIG.21

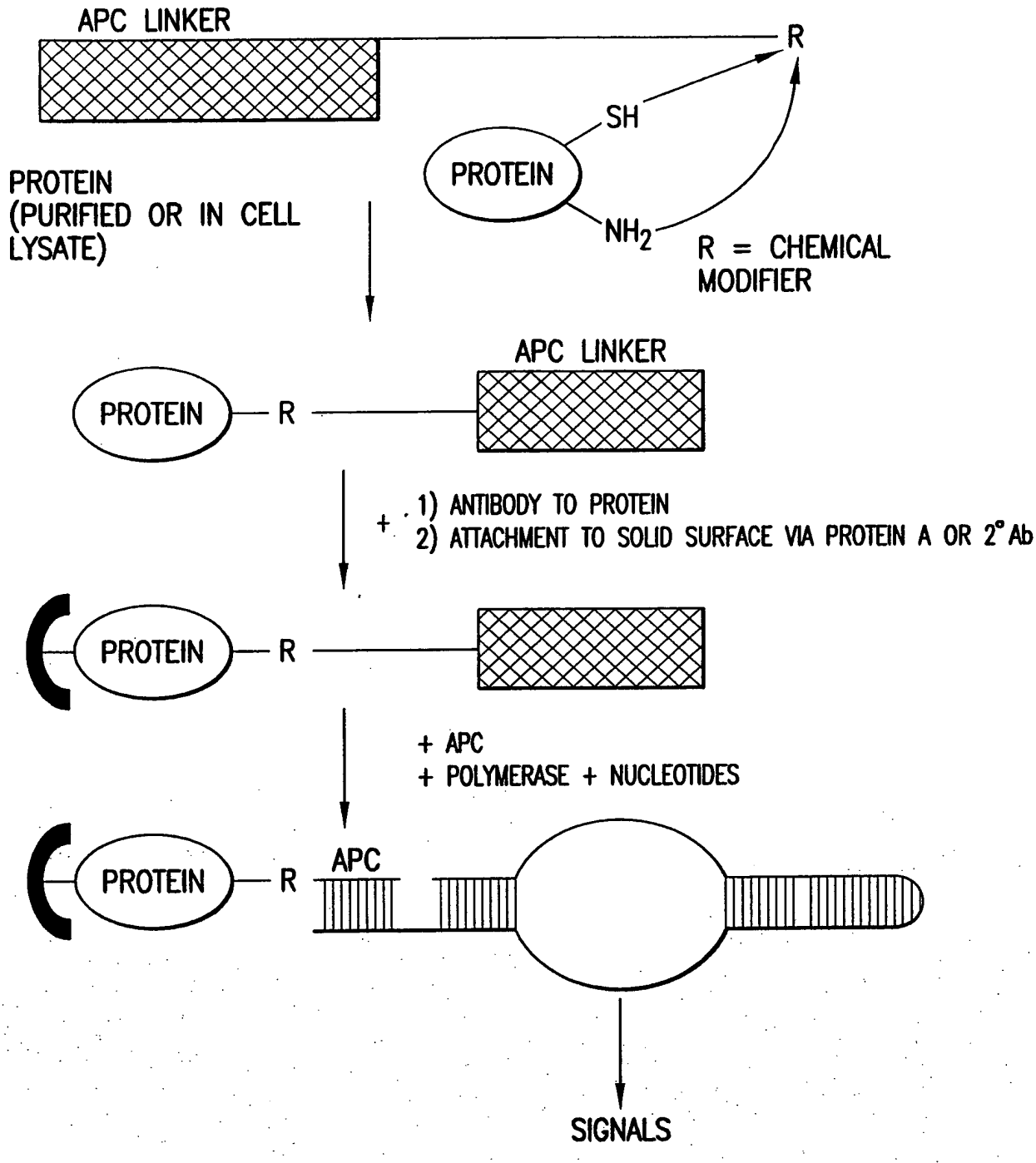

APC LINKER
R
SH
PROTEIN
NH2
PROTEIN (PURIFIED OR IN CELL LYSATE)
R = CHEMICAL MODIFIER
APC LINKER
PROTEIN
R
+ 1) ANTIBODY TO PROTEIN
2) ATTACHMENT TO SOLID SURFACE VIA PROTEIN A OR 2° Ab
PROTEIN
R
+ APC
+ POLYMERASE + NUCLEOTIDES
PROTEIN
R
APC
SIGNALS

FIG.22

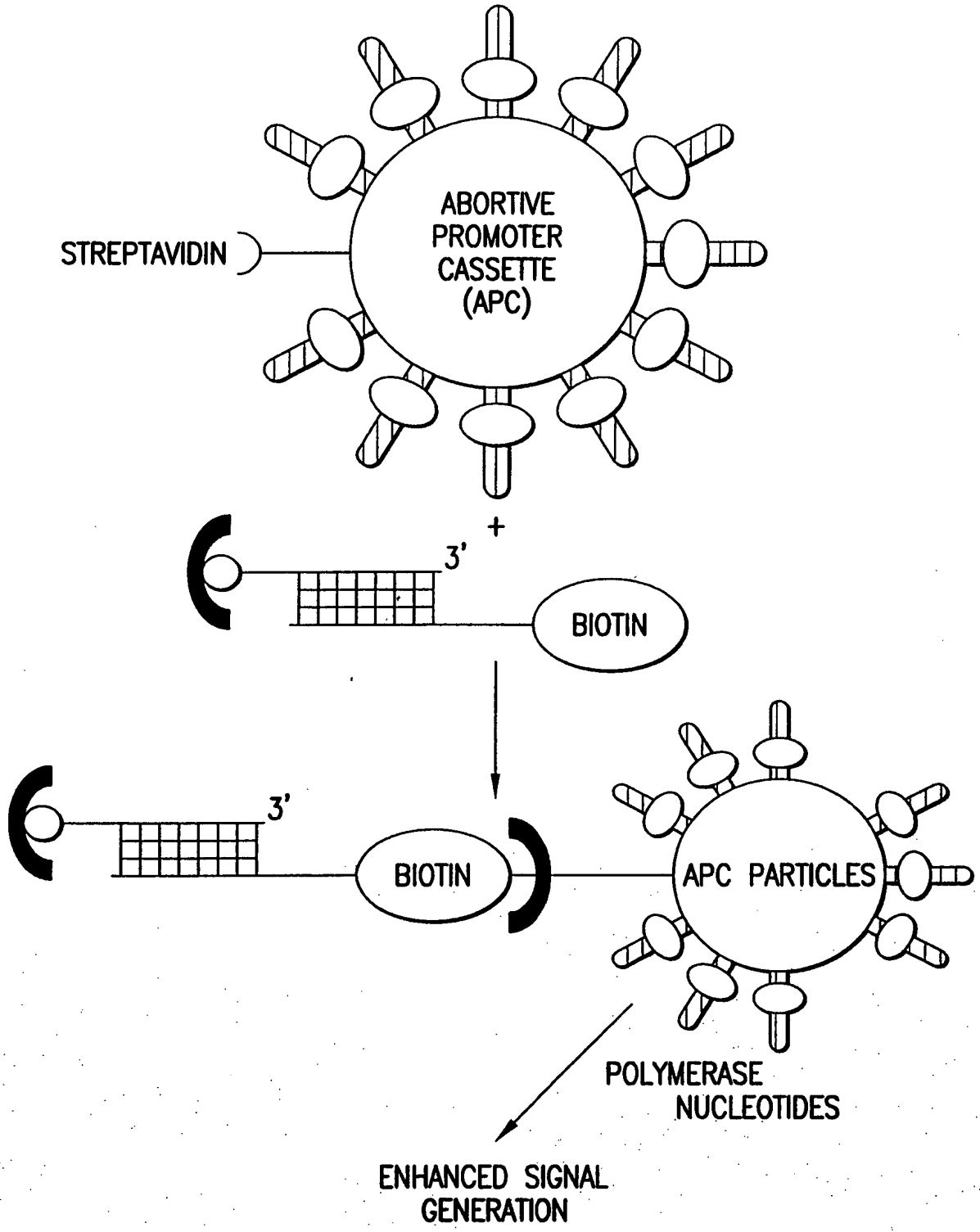

ABORTIVE PROMOTER CASSETTE (APC)
STREPTAVIDIN
+
3'
BIOTIN
3'
BIOTIN
APC PARTICLES
POLYMERASE NUCLEOTIDES
ENHANCED SIGNAL GENERATION

FIG.23

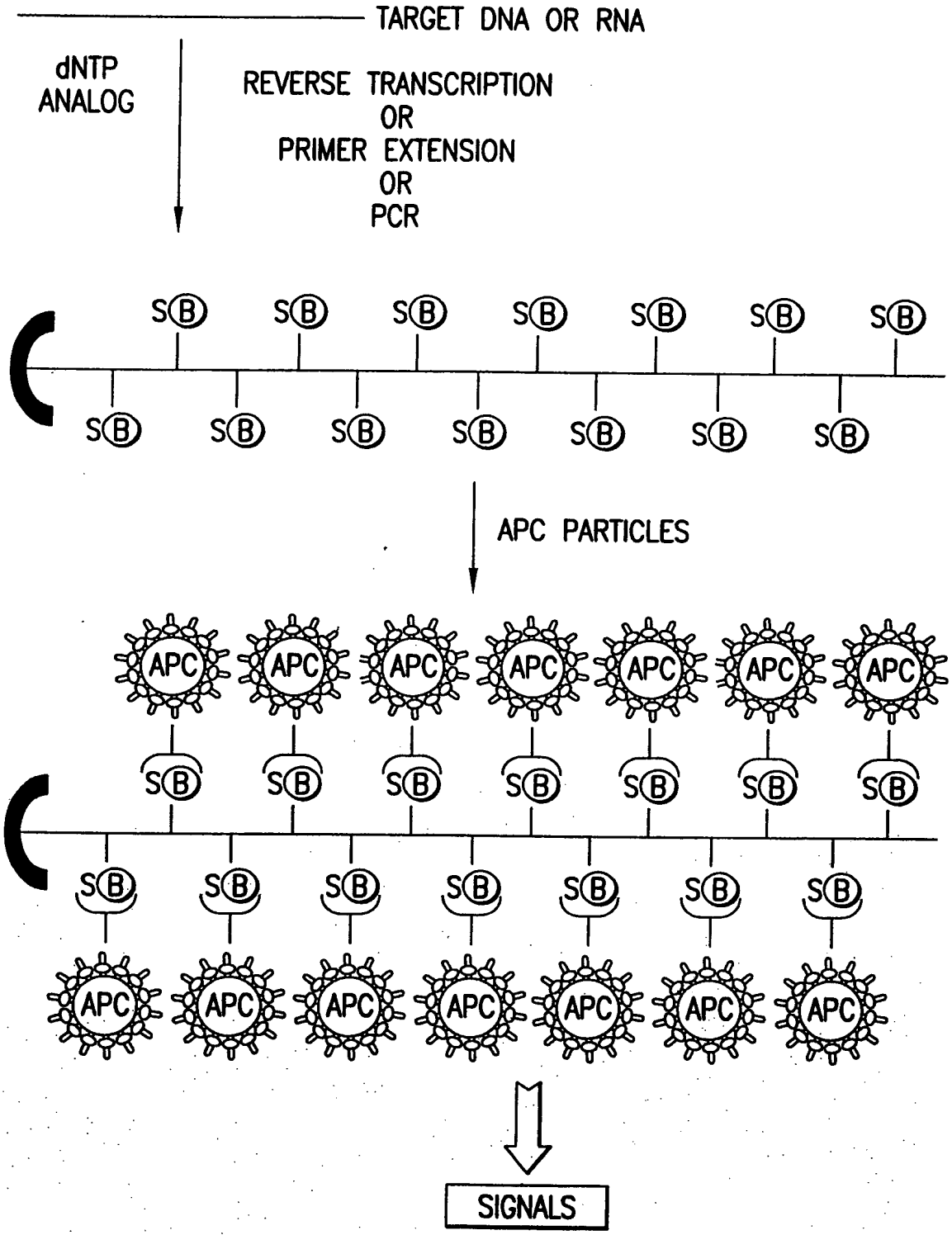

TARGET DNA OR RNA
dNTP ANALOG
REVERSE TRANSCRIPTION
OR
PRIMER EXTENSION
OR
PCR
S B
APC PARTICLES
APC
SIGNALS

FIG.24

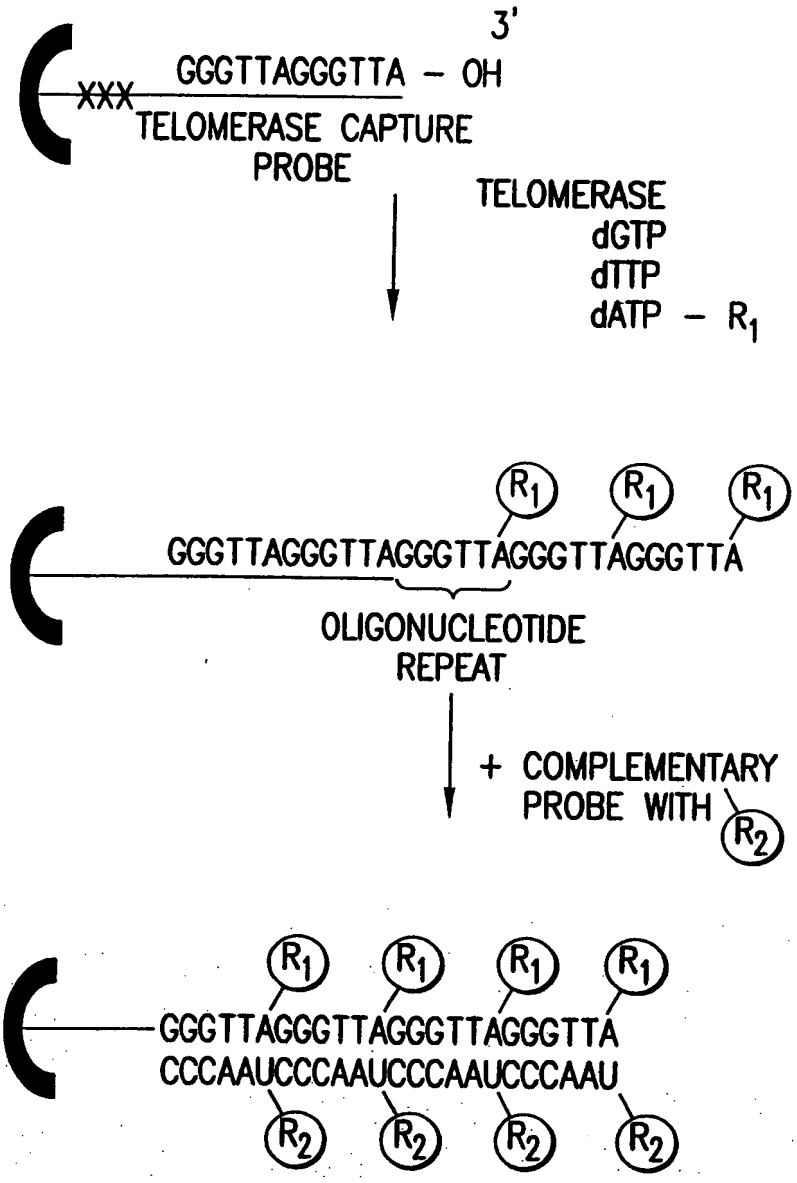
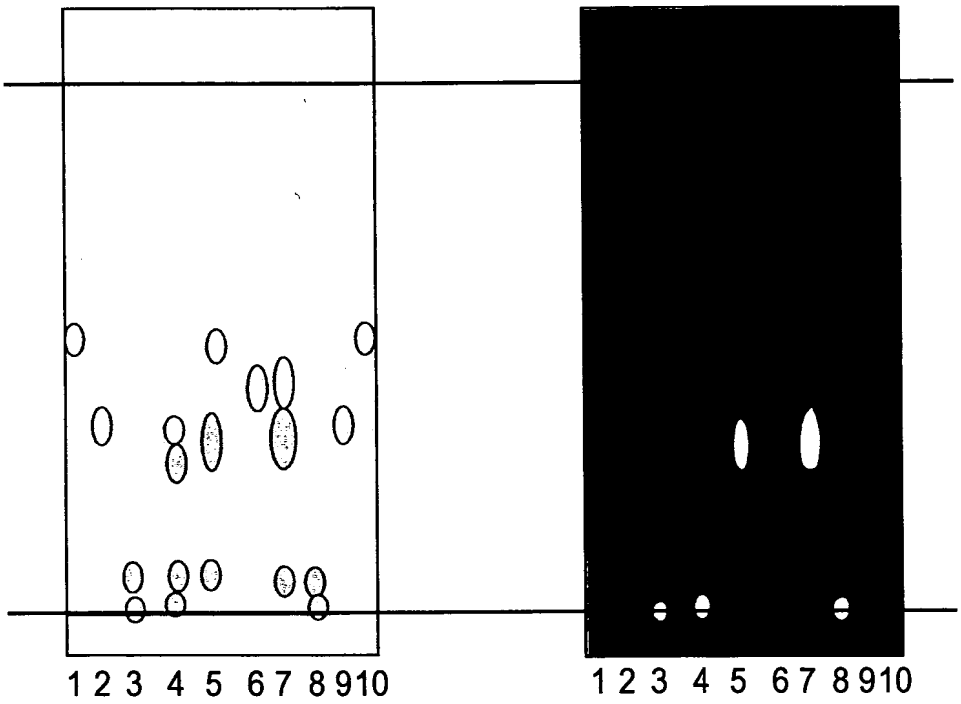

3'
GGGTTAGGGTTA – OH
XXX
TELOMERASE CAPTURE PROBE
TELOMERASE
dGTP
dTTP
dATP – R1
R1
R1
R1
GGGTTAGGGTTAGGGTTAGGGTTAGGGTTA
OLIGONUCLEOTIDE REPEAT
+ COMPLEMENTARY PROBE WITH
R2
R1
R1
R1
R1
GGGTTAGGGTTAGGGTTAGGGTTA
CCCAAUCCCAAUCCCAAUCCCAAU
R2
R2
R2
R2

FIG.25



Lane 1 CMPS
Lane 2 CTPS
Lane 3 IAEDANS
Lane 4 AEDANS-SpppC
Lane 5 AEDANS-S-pC
Lane 6 AMPS
Lane 7 AEDANS-SpA
Lane 8 IAEDANS
Lane 9 CTPS
Lane 10 CMPS

FIG.26

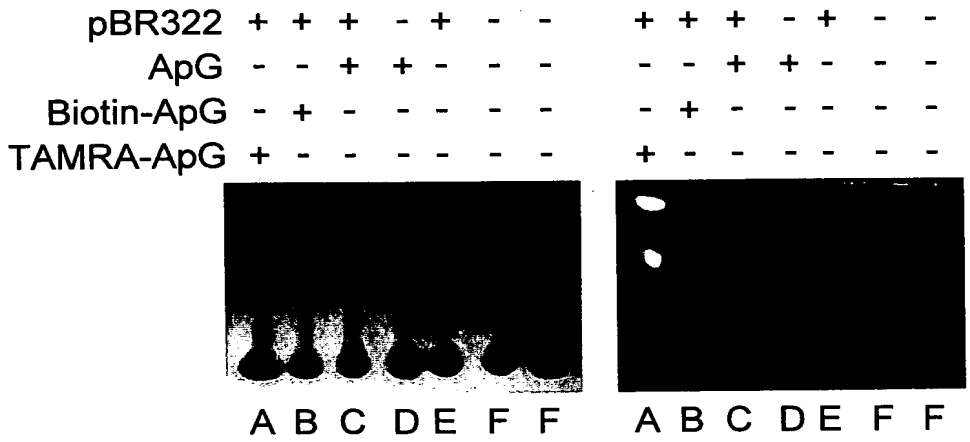


FIG.27

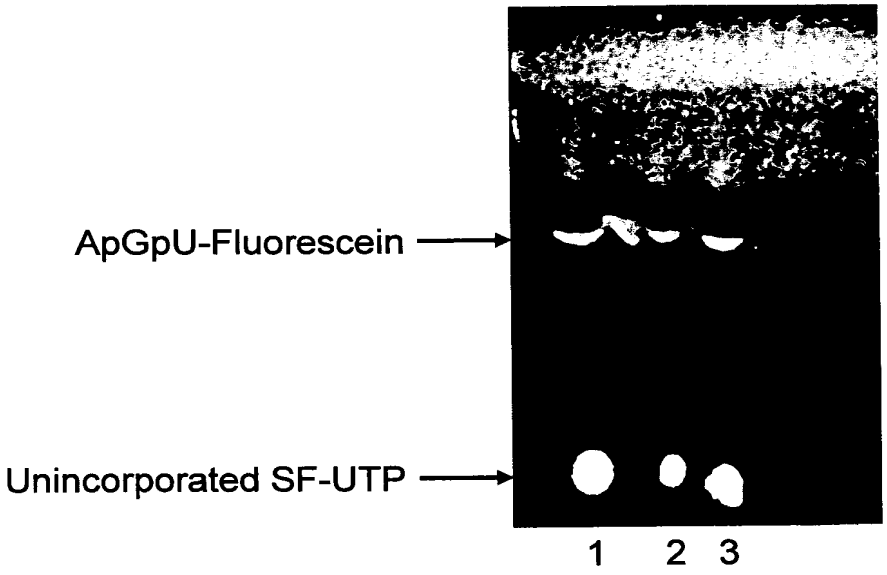


FIG.28

ATATACTGGGTCTACAAGGTTTAAGTCAACCAGGGATTGAAATATAACTTTTAAACAGAGCTGGATTATCCAGT
AGGCAGATTAAGCATGTGCTTAAGGCATCAGCAAAGTCTGAGCAATCCATTTTTTAAAACGTAGTACATGTTTT
TGATAAGCTTAAAAAGTAGTAGTCACAGGAAAAATTAGAACTTTTACCTCCTTGCGCTTGTTATACTCTTTAGT
GCTGTTTAACTTTTCTTTGTAAGTGAGGGTGGTGGAGGGTGCCCATAATCTTTTCAGGGAGTAAGTTCTTCTT
GGTCTTTCTTTCTTTCTTTCTTTCTTTTTTTCTTGAGACCAAGTTTCGCTCTTGTCTCCCAGGCTGGAGTGCAA
TGGCGCGATCTCGGCTCACTGCAACCTCCGCCTTCTCCTGGGTTCAAGCGATTCTCCTACATCAGCCTCCGA
GTAGCTGGGATTACAGGCATGCGCCACCAAGCCCCGCTAATTTTGTATTTTTTAGTAGAGACAGGGTTTCGC
CATGTTGGTCAGGCTTGTCTCGAACTCCTGGCCTCAGGTGATCCGCCTGTCTCGGCCTCCCAGAATGCTGG
GATTATAGACGTGAGCCACCGCATCCGGACTTTCCTTTTATGTAATAGTGATAATTCTATCCAAAGCATTTTTT
TTTTTTTTTGAGTCGGAGTCTCATTCTGTCACCCAGGCTGGAGGGTGGTGGCGCGATCTCGGCTTACTGCAA
CCTCTGCCTCCCGGGTTCAAGCGATTCTCCTGCCTCAGCCTCCTGAGTAGCTGGAATTACACACGTGCGCCA
CCATGGCCAGCTAATTTTTGTATTTTTAGTAGAGACGGGGTGTCACCATTTTGGCCAAGCTGGCCTCGAACTC
CTGACCTCAGGTGATCTGCCCGCCTCGGCTTCCCAAAGTGCTGGGATTACAGGTGTGAGCCACCGCGTCCT
GCTCCAAAGCATTTTCTTTCTATGCCTCAAAACAAGATTGCAAGCCAGTCCTCAAAGCGGATAATTCAAGAGC
TAACAGGTATTAGCTTAGGATGTGTGGCACTGTTCTTAAGGCTTATATGTATTAATACATCATTTAAACTCACA
ACAACCCCTATAAAGCAGGGGGCACTCATATTCCCTTCCCCCTTTATAATTACGAAAAATGCAAGGTATTTTC
AGTAGGAAAGAGAAATGTGAGAAGTGTGAAGGAGACAGGACAGTATTTGAAGCTGGTCTTTGGATCACTGTG
CAACTCTGCTTCTAGAACACTGAGCACTTTTTCTGGTCTAGGAATTATGACTTTGAGAATGGAGTCCGTCCTT
CCAATGACTCCCTCCCCATTTTCCTATCTGCCTACAGGCAGAATTCTCCCCCGTCCGTATTAAATAAACCTCA
TCTTTTCAGAGTCTGCTCTTATACCAGGCAATGTACACGTCTGAGAAACCCTTGCCCCAGACAGCCGTTTTAC
ACGCAGGAGGGGAAGGGGAGGGGAAGGAGAGAGCAGTCCGACTCTCCAAAAGGAATCCTTTGAACTAGGG
TTTCTGACTTAGTGAACCCCGCGCTCCTGAAAATCAAGGGTTGAGGGGGTAGGGGGACACTTTCTAGTCGTA
CAGGTGATTTCGATTCTCGGTGGGGCTCTCACAACTAGGAAAGAATAGTTTTGCTTTTTCTTATGATTAAAAGA
AGAAGCCATACTTTCCCTATGACACCAAACACCCCGATTCAATTTGGCAGTTAGGAAGGTTGTATCGCGGAG
GAAGGAAACGGGGCGGGGGCGGATTTCTTTTTAACAGAGTGAACGCACTCAAACACGCCTTTGCTGGCAGG
CGGGGGAGCGCGGCTGGGAGCAGGGAGGCCGGAGGGCGGTGTGGGGGGCAGGTGGGGAGGAGCCCAGT
CCTCCTTCCTTGCCAACGCTGGCTCTGGCGAGGGCTGCTTCCGGCTGGTGCCCCCGGGGGAGACCCAACC
TGGGGCGACTTCAGGGGTGCCACATTCGCTAAGTGCTCGGAGTTAATAGCACCTCCTCCGAGCACTCGCTC
ACGGCGTCCCCTTGCCTGGAAAGATACCGCGGTCCCTCCAGAGGATTTGAGGGACAGGGTCGGAGGGGGC
TCTTCCGCCAGCACCGGAGGAAGAAAGAGGAGGGGCTGGCTGGTCACCAGAGGGTGGGGCGGACCGCGT
GCGCTCGGCGGCTGCGGAGAGGGGGAGAGCAGGCAGCGGGCGGCGGGGAGCAGCATGGAGCCGGCGGC
GGGGAGCAGCATGGAGCCTTCGGCTGACTGGCTGGCCACGGCCGCGGCCCGGGGTCGGGTAGAGGAGGT
GCGGGCGCTGCTGGAGGCGGGGGCGCTGCCCAACGCACCGAATAGTTACGGTCGGAGGCCGATCCAGGT
GGGTAGAGGGTCTGCAGCGGGAGCAGGGGATGGCGGGCGACTCTGGAGGACGAAGTTTGCAGGGGAATT
GGAATCAGGTAGCGCTTCGATTCTCCGGAAAAAGGGGAGGCTTCCTGGGGAGTTTTCAGAAGGGGTTTGTA
ATCACAGACCTCCTCCTGGCGACGCCCTGGGGGCTTGGGAAGCCAAGGAAGAGGAATGAGGAGCCACGCG
CGTACAGATCTCTCGAATGCTGAGAAGATCTGAAGGGGGGAACATATTTGTATTAGATGGAAGTATGCTCTTT
ATCAGATACAAAATTTACGAACGTTTGGGATAAAAAGGGAGTCTTAAAGAAATGTAAGATGTGCTGGGACTAC
TTAGCCTCCAATTCACAGATACCTGGATGGAGCTTATCTTTCTTACTAGGAGGGATTATCAGTGGAAATCTGT

FIG. 29A

Appl. No. *To Be Assigned*; Group Art Unit: *To Be Assigned*; Inventors: Michelle M. Hanna.; Tel: 202.371-2600
Title: Molecular Detection Systems Utilizing Reiterative Oligonucleotide Synthesis

GGTGTATGTTGGAATAAATATCGAATATAAATTTTGATCGAAATTATTCAGAAGCGGCCGGGCGCGGTGCCTC
ACGCCTTGTAATCCCTTCACTTTGGGAGATCAAGGCGGGGGGAATCACCTGAGGTCGGGAGTTCGAGACCA
GCCTGGCCAACAGGTGAAACCTCGCCTCTACTAAAAATACAAAAAGTAGCCGGGGGGTGGTGGCAGGCGCCT
GTAATCCCAGCTACTCGGGAGGTTGAGGCAGGAGAATCGCTTGAACCCGGGAGGCTGAGGTTGTAGTGAAC
AGCGAGATGGAGCCACTTCACTCCAGCCTGGGTGACAGAGTGAGACTTTGTCGAAAGAAAGAAAGAGAGAA
AGAGAGAGAGAAAAATTATTCAGAAGCAACTACATATTGTGTTTATTTTTAACTGAGTAGGGCAAATAAATATA
TGTTTGCTGTAGGAACTTAGGAAATAATGAGCCACATTCATGTGATCATTCCAGAGGTAATATGTAGTTACCAT
TTTGGGAATATCTGCTAACATTTTTGCTCTTTTACTATCTTTAGCTTACTTGATATAGTTTATTTGTGATAAGAG
TTTTCAATTCCTCATTTTTGAACAGAGGTGTTTCTCCTCTCCCTACTCCTGTTTTGTGAGGGAGTTAGGGGAG
GATTTAAAAGTAATTAATACATGGGTAACTTAGCATCTCTAAAATTTTGCCAACAGCTTGAACCCGGGAGTTTG
GCTTTGTAGTCCTACAATATCTTAGAAGAGACCTTATTTGTTTAAAAACAAAAAGGAAAAAGAAAAGTGGATAG
TTTTGACAATTTTTAATGGAG

FIG. 29B

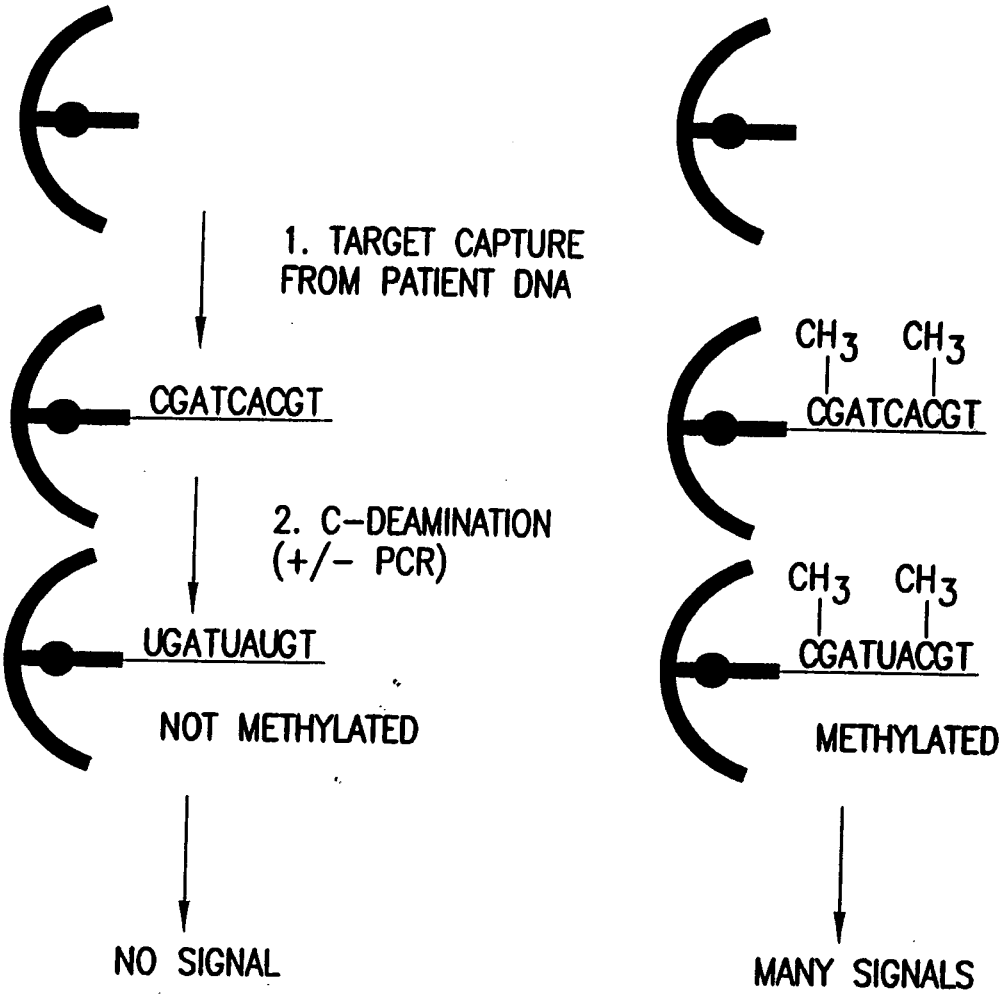

1. TARGET CAPTURE FROM PATIENT DNA
CGATCACGT
2. C-DEAMINATION (+/- PCR)
UGATUAUGT
NOT METHYLATED
NO SIGNAL
CH3 CH3
CGATCACGT
CH3 CH3
CGATUACGT
METHYLATED
MANY SIGNALS

FIG. 30